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Report of the Biotechnology Science Advisory Committee of the U.S. Environmental Protection Agency

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Contents

Introduction

In July of 1986 the U.S. Environmental Protection Agency (EPA) announced* its intention to form an advisory committee for biotechnology. This committee, the Biotechnology Science Advisory Committee (BSAC), was duly formed in December of 1986, and held its first meeting on April 27, 1987.

Because of great interest in the biotechnology arena and to foster communication within the Agency, summaries of meetings will be provided. These summaries will be included in the "Report of the Biotechnology Science Advisory Committee." This first "Report of the Biotechnology Science Advisory Committee" contains the charter under which the BSAC was formed and its current roster. Reports from two BSAC subcommittees to the BSAC are included, as well as the most recent draft version of the "EPA Guidelines for Contained Greenhouses." These three items were received and accepted as working documents by the BSAC in its April 27, 1987 meeting.

These documents are "working documents" which EPA is using in its attempt to develop definitions necessary to the implementation of its biotechnology policy. They are not to be considered as final documents, and it should be recognized that the BSAC and its subcommittees are advisory to the Agency.

Communications concerning the "Report of the Biotechnology Science Advisory Committee" should be addressed to the Executive Secretary, Biotechnology Science Advisory Committee, U.S. Environmental Protection Agency, 401 M St. S.W., Washington, D.C. 20460, Mail Code TS-788.

Executive Secretary
Biotechnology Science Advisory Committee

*Federal Register, July 2, 1986, Volume 51:24221

Biotechnology Science Advisory Committee
Advisory Committee Charter

1. **Purpose and Authority.** This Charter establishes the EPA Biotechnology Science Advisory Committee in accordance with requirements of the Federal Advisory Committee Act, 5 U.S.C. (App. I) 9(c), to meet the needs for specialized support for Agency consideration of biotechnology issues and to respond to the mandate for "agency-based scientific advisory committees" appearing in the Office of Science and Technology Policy announcement of December 31, 1984 (49 F.R. 50905) and November 14, 1985 (50 F.R. 47174).

2. **Scope of Activity.** The activities of the Committee will include analyzing problems, conducting reviews, holding meetings, providing reports, making recommendations, forming study groups, and other activities needed to meet the Committee's objectives, including the use of consultants as necessary.

3. **Objectives and Responsibilities.** The Committee will provide expert scientific advice to the Administrator and Assistant Administrators concerning issues relating to risks and other effects of applications of modern biotechnology. The committee shall provide reports and recommendations directly to the Administrator and to the Assistant Administrator(s) and will do so in a timely manner. The Committee responsibilities will include:

- Consideration of scientific issues referred by Program Office Directors;
- Comparison of case reviews to evaluate internal scientific consistency among programs;
- Assessment, in participation with the Science Advisory Board, of issues requiring research and referral to appropriate Agency research committees;
- Recommendation of issues to be referred to interagency coordinating committees through appropriate delegates;
- As appropriate, participation in review and evaluation of specific regulatory applications and submissions;
- As appropriate, consultation and coordination with the FIFRA Scientific Advisory Panel established by the Administrator pursuant to section 25(d) of the Federal Insecticide, Fungicide, and Rodenticide Act as amended;
- As appropriate, consultation and coordination with the Science Advisory Board established by the Administrator pursuant to the Environmental Research, Development and Demonstration Authorization Act of 1978;
- Consultation and coordination with other Agency advisory groups, as requested by the Administrator.

4. **Membership.** The Committee will consist of 11 voting members, including 9 scientists and 2 persons representing the general public. Subcommittees must include at least one member of the full Committee. The Administrator will appoint from the membership a Chairperson of the full Committee. The Administrator or the Administrator's designee will appoint Chairpersons of subcommittees or panels as needed, after consultation with the Chairperson. The Committee will be supplemented by consultants when they are needed to extend the range of expertise and experience of the standing Committee.

Scientist members of the Committee will be selected on the basis of their professional qualifications to examine the questions of hazard, exposure and risk to humans, other non-target organisms and ecosystems or their components due to production and release of organisms for purposes regulable under statutes for which the Environmental Protection Agency has responsibility.

As a minimum, the Committee shall have one scientist member who also serves as a member of the Administrator's Science Advisory Board Executive Committee. Other members can also have joint membership on this Committee and the Science Advisory Board or its various committees or study groups.

As a minimum, the Committee shall have one scientific member who also serves as a member of the FIFRA Scientific Advisory Panel. Other members can also have joint membership on this Committee and the Scientific Advisory Panel or its Subpanels.

In addition, there will be nonvoting representatives from each Federal agency represented on the Biotechnology Science Coordinating Committee of the Federal Coordinating Council for Science, Engineering and Technology.

The Committee is authorized to form subcommittees or panels for any purpose consistent with this charter. The Administrator or the Administrator's designee shall review the need for such subcommittees and panels at least yearly to decide which should be continued. The subcommittees and panels will operate under the direction of the Committee.

5. **Meetings.** The Committee will meet at the request of the Administrator or the Administrator's designee. Meetings will be called, announced, and held in accordance with the EPA Manual on Committee Management. The Manual provides for open meetings of advisory committees; requires that interested persons be permitted to file written statements before or after meetings; and provides for oral statements by interested persons to the extent time permits. Meetings or portions thereof may be closed to comply with statutory restrictions concerning dissemination of proprietary and confidential information; however, the Agency is committed to having open meetings to the greatest extent possible. A full-time salaried officer or employee of the Agency, who will be designated as Executive Secretary, will be present at all meetings and is authorized to adjourn any such meeting whenever it is determined to be in the public interest.

It is anticipated that the full Committee will meet approximately three times per year, supplemented by subcommittee meetings as needed. The estimated annual operating costs for the Committee will be approximately $125,000 which includes 1.0 work-year of staff support.

Support for the Committee's activities will be provided by the Office of the Administrator, EPA or other appropriate offices as necessary.

6. **Duration.** The Committee will function for two years, and may continue after the two years if needed. The continuing need for the Committee will be re-evaluted at the end of the first year.

Biotechnology Science Advisory Committee
Announcement of Committee Members

Summary
On July 2, 1986, the EPA announced its intent to prepare a list of candidates from which nominees would be selected for the Biotechnology Science Advisory Committee (BSAC) and/or its Subcommittees. A list of such candidates was prepared and from that list were selected individuals to form the BSAC. The BSAC was established to provide expert scientific advice to the EPA Administrator concerning issues relating to applications of modern biotechnology.

"Supplementary Information"
Members of the Biotechnology Science Advisory Committee and their backgrounds are:

Rita Colwell, Chairperson: B.S. Purdue University, M.S. Purdue University, Ph.D. University of Washington. Vice-President of Academic Affairs, and Professor of Microbiology, University of Maryland. Research Interests: Marine biotechnology, marine and estuarine microbial ecology; survival of pathogens in the aquatic environment, microbial biodegradation. Committees: EPA Environmental Research Board, appointed 1984; National Science Foundation, Biotechnology Committee, January 30, 1984; Natural Resources Defense Council, Diversity Task Force, 1982-present; International Cell Research Organization, 1979-present.

Robert Colwell: A.B. Harvard College, Ph.D. University of Michigan. Professor, University of California, Berkeley, Department of Zoology. Research Interests: Community biology; species interaction and coevolution, species diversity and biogeography, patterning in space and time, adaptive morphologies and life histories and biological systematics. Committees: NIH RAC Working Group on Release into the Environment, 1984-present; Ad Hoc Consultant to EPA and USDA, 1984-present.

Douglas Rouse: B.S. Ottawa University, M.S. Colorado State University, Ph.D. Pennsylvania State University. Associate Professor, University of Wisconsin. Research Interests: Plant pathology, mathematical modeling and quantitative analysis, population dynamics, use of biocontrol agents in the field, practical plant breeding experience.

David Stahl: B.S. University of Washington, M.S. University of Illinois, Ph.D. University of Illinois. Assistant Professor of Veterinary Microbiology, Department of Veterinary Pathobiology, University of Illinois, Urbana. Research Interests: Molecular approaches to microbial ecology, molecular phylogeny of microorganisms, ribosomal RNA processing.

James Tiedje: B.S. Iowa State University, M.S. Cornell University, Ph.D. Cornell University. Assistant, Associate and Professor, Departments of Crop and Soil Sciences and Public Health, Michigan State University, 1968-present. Acting Director for Development of Research, Michigan Agricultural Experiment Station, 1977-78. Research Interests: Role of terrestial (and aquatic) bacteria on nitrogen, sulfate, carbon, phosphate and hydrogen cycles, microbial degradation of xenobiotic chemicals. Committees: USDA Competitive Grants Review Panel, 1980-81. FIFRA Scientific Advisory Panel.

Richard Merrill: A.B. Columbia College, B.A. Oxford University, M.A. Oxford University, LL.B. Columbia University School of Law. University of Virginia School of Law: Associate Professor, 1969-72; Professor, 1972-77; Associate Dean, 1974-75; Daniel Caplin Professor, 1977-85; Arnold Leon Professor, 1985-present; Dean, 1980-present. Chief Counsel, U.S. FDA, 1975-77. Committees: Council of the Institute of Medicine, National Academy of Sciences; IOM Member, 1977-Present.

Ralph Mitchell: B.A. Trinity College, Dublin, Ireland, M.S. Cornell University, Ph.D. Cornell University. Gordon McKay Professor of Applied Biology, Division of Applied Sciences, Harvard University, 1970-Present. Research Interest: Industrial microbiology. Committees: National Research Council, National Committee on Water Quality, American Society of Microbiology, Committee on Environmental Hazards.

Charles Hagedorn: B.S. Kansas State University, M.S. Iowa State University, Ph.D. Iowa State University. Professor of Agronomy and Plant Pathology, Virginia Polytechnic University, 8/86-present. Manager and Senior Microbiologist, Crop Science Laboratory, Allied Corporation, 9/83-7/86. Research Interests: Environmental Microbiology, Microbial Ecology, Soil, Aquatic and Agricultural Microbiology. Committees: Member, Review Panel, EPA Biotechnology Risk Assessment Program, effective 12/10/85. Member, Environmental Chemistry and Physics Review Panel, EPA, Columbus, Ohio 1980-83.

Jay Hair: B.S. Clemson University, M.S. Clemson University, Ph.D. University of Edmonton, Canada. Research and Management Consultant, South Carolina Wildlife and Marine Resources Department, 1976-77. Assistant Professor of Wildlife Biology, Clemson University, 1973-77. Special Assistant, U.S. Department of Interior, Office of the Assistant Secretary, Fish and Wildlife and Parks, 1978-80. Associate Professor of Zoology and Forestry, 1977-81. Executive Vice-President, National Wildlife Federation, 1981-present. Committees: American Association for the Advancement of Science; American Forestry Association; Association of University Fisheries and Wildlife Program Administrators; Ecological Society of America; International Association of Fish and Wildlife Agencies; Wildlife Society; Nature Conservancy.

Susan Gottesman: B.S. Radcliffe College, Ph.D. Harvard. Acting Chief, Biochemical Genetics Section, Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health. Research Interests: Global control of gene expression in gram negative bacteria;

genetic control of bacterial cell growth; regulation of proteolysis in *E. coli;* site specific recombination. Committees: RAC Risk Assessment Subcommittee, RAC Phage Working Group; RAC Human Gene Therapy Working Group; RAC Working Group on Release to the Environment; RAC Working Group on Revision of the Guidelines.

Francis Macrina: B.S. Cornell University, Ph.D. Syracuse. Professor and Chairman, Department of Microbiology and Immunology, Virginia Commonwealth University. Research Interests: Antibiotic resistance in oral and intestinal flora. Committees: EPA Science Advisory Board: Study Group on Biotechnology 1985-86, NIH RAC Working Group on Gram Positive Bacteria; Consultant, FDA, use of antibiotics in animal feed.

EPA Biotechnology Science Advisory Committee

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The Approach of the U.S. Environmental Protection Agency in Regulating Certain Biotechnology Products

The U.S. Environmental Protection Agency functions under a number of statutes to carry out its mission of protecting human health and the environment. While these statutes were not written specifically for biotechnology or products of biotechnology, there are two which have been interpreted as investing EPA with the authority to do so. These are the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) and the Toxic Substances Control Act (TSCA).

FIFRA creates a statutory framework under which EPA regulates, by a registration statute, the sale and distribution of pesticides. A pesticide will be registered for use only if EPA determines that the pesticide will not cause or increase the risk of unreasonable adverse effects to humans or the environment. In order to register a pesticide, an applicant must submit or cite to EPA data on subjects such as product composition, toxicity, environmental fate, and effects on nontarget organisms. This process involves premarket review of data on a pesticide's safety, and regulation of the use of a pesticide before it is registered, since some of the data needed for a registration review must be developed in field tests under actual use conditions. In order to test in the field, an applicant may obtain an experimental use permit authorizing limited use of an unregistered product. Such a permit may be issued if the proposed experiment will generate data needed to register a pesticide and the experiment will not cause unreasonable adverse effects on the environment.

FIFRA gives EPA oversight responsibility over microbial pest control agents whether or not these agents are genetically engineered. The fact that EPA has the authority to approve pesticides for testing or use as a prerequisite for entry into the environment is a powerful regulatory tool. FIFRA places on the registrant or applicant the burden of proof that the benefits of use of the product outweigh the risks.

TSCA authorizes EPA to acquire information on "chemical substances" and "mixtures" of chemical substances in order to identify potential hazards and exposures. EPA can regulate the production, processing, distribution, use and disposal of chemical substances if they present an unreasonable risk of injury to health or the environment.

Under TSCA, EPA can also require testing of any "chemical substance" that may present an unreasonable risk of injury to health or the environment or is produced in substantial quantities and may result in substantial environmental release or substantial human exposure.

TSCA gives EPA jurisdiction over the manufacture, processing, distribution, use, and disposal of all chemicals in commerce or intended for entry into commerce that are not specifically covered by other regulatory authorities (foods, drugs, cosmetics, pesticides, etc.). Thus, TSCA serves as "gap filling" environmental law. It is concerned with all exposure media (air, water, soil, sediment, biota).

The heart of TSCA is section 5, which implements the act's goal of identifying potentially hazardous new chemical substances before they enter commerce. This section requires that manufacturers and importers of "new" chemical substances intended for commercial use submit a premanufacture notice to EPA at least 90 days before manufacture or import is to begin. EPA has 90 days to prohibit or regulate the production, processing, distribution or import and disposal of a "new" substance, otherwise manufacture or import may begin. The Agency review period can be extended to 180 days if necessary for good cause. The manufacturer or importer must submit information as specified in the notification form, and which is either "known or reasonably ascertainable" at the time of submission. They must also submit test data that are in their possession and under their control at the time of submission.

EPA is using various statutory mechanisms under FIFRA and TSCA to ensure that it receives reports in advance of the first test release of any particular microorganism which falls under EPA purview.

TSCA's applicability to regulating microbial biotechnology products is based on the interpretation that microbes are chemical substances under TSCA (OSTP). Under TSCA, microorganisms which through deliberate human intervention were altered to contain genetic material from dissimilar source organisms are considered "new" and subject to premanufacture notification (PMN) requirements under section 5 if they are manufactured or imported for TSCA purposes. EPA defines "dissimiliar" as organisms from different taxonomic genera; the organisms resulting from such a combination are called "intergeneric."

In addition to the section 5 PMN requirements, two other provisions of TSCA provide key elements for oversight of microorganisms produced by the biotechnology industry. These are the section 5 significant new use authority and the section 8 reporting authority.

Recognizing that some microorganisms or applications may present less concern than others, the EPA has constructed a system with two different levels of review; more information will be evaluated in one level of review than in the other and the review period will differ.

EPA at present intends to focus on three categories of microorganisms for closer regulatory attention. The three types of microorganisms that will receive the greatest regulatory emphasis are microorganisms with "new" characteristics or that are new to the environment in which they will be used (and whose behavior is therefore less easily predictable), microorganisms that have pathogenic attributes (pathogens or organisms containing genetic material from pathogens), and microorganisms that are used in the environment (and therefore have the potential for widespread exposure).

EPA will implement this policy under TSCA in several ways: (1) In order to require that a PMN review be conducted prior to the environmental release of any "new" microorganism, the Agency will amend an existing rule

so that environmental releases in the course of "research and development" on a microorganism will not be exempt from PMN review as they are under the current PMN rule. (2) Under section 5(a)(2) of TSCA, the Agency will issue a significant new use rule (SNUR) which will cover certain microorganisms that are pathogens or have been deliberately altered to contain genetic material from pathogens. (3) All other microorganisms which are to be deliberately released to the environment will be subject to reporting requirements under TSCA section 8(a). Section 8(a) reporting requirements will specify fewer information requirements than a section 5 PMN or SNUR review will specify. EPA is requesting that industry voluntarily comply with the first two of these policies until they are actually implemented by the Agency.

As part of the experimental use permit process, EPA has developed special procedures for genetically engineered and nonindigenous microorganisms.

EPA's current policy for small scale field testing of microorganisms subject to FIFRA also provides for two levels of notification and review. The three categories of microorganisms that EPA has determined will receive the greatest regulatory emphasis will be subject to 90-day notification and review to determine if an experimental use permit is required. There is also an expedited, abbreviated, 30-day review for small scale testing of all other genetically engineered or nonindigenous microbial pesticides. At the end of the 30-day review, EPA will determine whether or not an EUP is required, or whether the experiment may proceed without further review.

Those parts of the policy under FIFRA requiring rulemaking would formally codify, as part of the Experimental Use Permit (EUP) Regulations, the Interim Policy Statement issued in October 1984 which eliminated the exemption for field tests involving less than 10 acres of land and one acre of water for genetically engineered pesticides.

For the immediate future, EPA will review submissions received under FIFRA and TSCA on a case-by-case basis. EPA will incorporate both review by EPA scientists and review by recognized experts into the process in order to obtain the best scientific advice available. EPA emphasis on external scientific peer review is noteworthy. Recognized scientists possessing expertise pertinent in the review of genetically engineered organisms (including topic areas such as ecology, microbial ecology, molecular genetics, physiology, etc.) have been consulted on an *ad hoc* basis. Included in this group are members of the EPA Scientific Advisory Panel, a statutory advisory group established to provide external review and comment on scientific issues related to the regulation of pesticides.

As part of EPA's efforts to obtain the best scientific advice available, EPA has instituted a Biotechnology Science Advisory Committee (BSAC). This committee, which is advisory to the Administrator of the EPA, is composed of 11 voting members: nine scientists and two representatives of the public. The BSAC will analyze problems, conduct reviews, offer recommendations to the Agency and help meet the EPA need for state-of-the-art scientific advice on the issues of biotechnology. Scientist members of the BSAC have been selected on the basis of their professional qualifications to examine questions of hazard, exposure, and risk to humans, other nontarget organisms, and ecosystem or ecosystem components. The BSAC will be supplemented by consultants when they are needed to extend the range of expertise and experience of the standing committee.

Issues to be Resolved for TSCA Biotechnology Rules

R&D Rule

Definition of "environmental release"

Definition of "commercial research"

SNUR

Definition of "environmental release"

Organisms to be subjected to the SNUR

Reporting Rule

Definition of "environmental release"

Definition of "small manufacturer or processor"

What information to report

Report of the Meeting of the Biotechnology Science Advisory Committee; Subcommittee on Definition of Release Into the Environment

December 11-12, 1986

Introduction

The use of the techniques which have been called the "new" biotechnology (recombinant DNA, cell fusion, etc.) has brought to the fore certain issues in assessing the potential impacts of the technology. Among these issues are the definition of what constitutes "contained" and what constitutes "released" to the environment when microorganisms are used to perform certain tasks.

In general, "contained" has been used to mean procedures that occur within a facility where certain equipment, procedures and practices are employed to "control" microorganisms. Few facilities, however, ever provide absolute containment; in practice, varying numbers of microorganisms are released when microorganisms are used in facilities such as laboratories or fermentation plants. A number of factors affect the quantities of viable organisms released from a facility during a specific process. Thus, no absolute standard exists for "contained" or for "released." Yet for the purposes of understanding and describing the effect of adding microorganisms to environments, and to regulate microbial products, workable definitions of "contained" and "released" are necessary.

In order to obtain advice in its efforts to develop a workable definition of "released", the Environmental Protection Agency (EPA) has assembled a group of recognized technical experts as a subcommittee of an Agency-based scientific advisory committee. Those experts met on December 11-12, 1986, at Crystal Mall-2, in Crystal City, VA. This report describes that meeting.

A brief review of the history of that technique of the "new" biotechnology known as recombinant DNA is included in this report to illustrate how the concepts of "contained" and "released" have influenced the oversight of recombinant DNA technology. The report also describes the format and objectives of the December 11-12 meeting, and includes the minutes and a roster of the experts who participated in the meeting. Most importantly, the report details the several approaches to defining "contained" and "released" suggested by the subcommittee.

History

It was the scientists[1] who engaged in research using the technique called recombinant DNA who first called for assessment of the risks which might be associated with this technique.

At an international meeting of scientists in February 1975 at the Asilomar Conference Center, Pacific Grove, CA, the participants agreed recombinant DNA experiments should proceed, provided appropriate physical and biological containment* is utilized.

The National Institutes of Health (NIH) was asked[2] to develop (1) guidance on appropriate physical and biological containment, and (2) the mechanism to evaluate experiments in order to assign appropriate containment.

In July 1976, NIH published its Guidelines for Research Involving Recombinant DNA Molecules[3]. These guidelines, which were developed with the assistance of the Recombinant DNA Molecules Advisory Program (later renamed the Recombinant DNA Advisory Committee [RAC]), were based upon containment of organisms containing recombinant DNA.

In this original version of the guidelines, intentional release to the environment of organisms constructed using this technique of the "new" biotechnology was considered a special category: experiments involving "deliberate release into the environment of any organism containing a recombinant DNA molecule" were "not to be initiated. . . ."

In the first revision of the NIH Guidelines in 1978[4] this specification was modified to language which prohibited "deliberate release into the environment of any organism containing recombinant DNA"; individual waivers could, however, be approved by the Director, NIH, following publication of proposals for public comment in the *Federal Register* and review by the NIH RAC and approval by the local oversight committee, the Institutional Biosafety Committee (IBC). In the April 1982 version[5] of the guidelines, this language was modified to permit experiments involving release to the environment but NIH review and NIH and IBC approval before initiation are required.

In these various versions of its guidelines, the NIH did not officially define what constitutes release into the environment. In general, release was considered to be any experiment or procedure which occurred outside of a contained facility such as a laboratory or a fermentation plant in which equipment, practices, and procedures described in the NIH guidelines were employed.

Eventually, beginning in 1983 NIH developed language which was to serve as guidance for certain testing involving modified organisms outside of "contained" facilities. These documents describe the types of data required for evaluating experiments involving field testing of: (1) certain modified plants[6], and (2) modified microorganisms.[7] These documents do not specifically define "release."

As biotechnology products approached the point of testing in the environment, concerns were raised about the adequacy of the NIH system to oversee these products of the "new" biotechnology. These concerns arose for several reasons:

- The NIH Guidelines only apply to those institutions which receive government funding. They do not apply to

*Biological containment depends on characteristics the organisms possess which limit their ability to survive. Physical containment consists of a set of equipment, operating procedures and practices, and facility design (buildings). The goal of containment is to "control" or "restrict" dissemination of the organisms.

industry although the NIH had developed a system of voluntary compliance for institutions not receiving government funds.

- The NIH does not have any special system for protecting confidential business information.

- As the number of proposed applications increased there was concern that the number of applications would be large and place a large burden on NIH.

Therefore, in the spring of 1984, the government formed an interagency working group under the White House Cabinet Council on Natural Resources and the Environment (the Cabinet Council Working Group has since been reorganized under the Domestic Policy Council). It was the consensus of the group that various agencies (United States Department of Agriculture [USDA], Food and Drug Administration [FDA] and EPA) have the necessary authority to regulate the products of biotechnology under existing statutes; and the government constructed a coordinated regulatory framework of several agencies (USDA, EPA, FDA, NIH and National Science Foundation) to regulate biotechnology[8]

Two EPA statutes are currently applied to biotechnology products among those statutes believed to invest EPA with the authority to regulate products of biotechnology. The statutes used at this time to regulate biotechnology products are:

- The Federal Insecticide, Fungicide and Rodenticide Act (FIFRA)[9] which creates a regulatory framework under which EPA regulates the sale and distribution of pesticides. FIFRA gives EPA oversight responsibility over microbial pest control agents whether or not these agents are genetically engineered.

- The Toxic Substances Control Act (TSCA)[10] authorizes EPA to acquire information on "chemical substances" and "mixtures" of chemical substances in order to identify potential hazards and exposures. TSCA gives EPA jurisdiction over the manufacturing, processing, distribution, use and disposal of all chemicals in commerce or intended for entry into commerce that are not specifically covered by other regulatory authorities. TSCA's applicability to regulating microbial biotechnology products is based on the interpretation that microbes are chemical substances under TSCA.

In the June 26, 1986, *Federal Register*[8], EPA detailed the applicability of TSCA and FIFRA to certain living microorganisms. That *Federal Register* also:

- made certain policies immediately effective and others voluntary. The policies which are currently voluntary will be made effective by rulemaking.

- indicated that a definition of release will be developed through the rulemaking process. In the interim, the Agency is relying on a definition of release that focuses on containment; i.e., procedures that are not "contained" as defined in the document are considered "release."

At this time, three rules must be promulgated under TSCA in order to fully implement EPA's biotechnology policy. A definition of "release into the environment" is needed to circumscribe the scope of these three rules. These rules are:

- In order to require that a Premanufacture Notice (PMN) review be conducted prior to the release of any microorganism covered by the policy, the Agency will amend the section 5 rule so that environmental releases in the course of "research and development" will not be exempt from PMN review.

- Under section 5(a)(2) of TSCA, EPA will issue a significant new use rule (SNUR) which will cover certain microorganisms which are pathogens or have been deliberately altered to contain DNA from pathogens. Environmental uses of these organisms would be considered "significant new uses."

- EPA will issue a rule under section 8(a) of TSCA to require reporting of information about organisms and uses. This will provide a mechanism of monitoring the industry and ensuring an increasing base of information with which to assess and modify regulatory decisions. This rule would cover environmental uses.

In addition to the need for a definition of release to fully implement the TSCA statutes, enforcement of the biotechnology policy under FIFRA also depends on a definition of "release" to the environment.

Applicability of Definition

A definition of release is needed to permit both the Agency and industry to determine whether a particular use of a microbial product constitutes a release to the environment.

The definition of release will apply to environmental applications of microbial pesticides under FIFRA and to environmental releases of microorganisms subject to TSCA.

Under TSCA, all microorganisms produced for environmental, industrial, or consumer uses are potentially regulable with the exception of microorganisms used as foods, food additives, drugs, cosmetics, medical devices, and pesticides and microorganisms used to produce foods, food additives, drugs, cosmetics, and medical devices. It is not possible to list all the applications that could be subject to TSCA because many will undoubtedly be developed in the future.

Examples of the types of microorganisms that would be subject to TSCA include microorganisms used in conversion of biomass for energy, pollutant degradation, sewage treatment, enhanced oil recovery, fossil fuel desulfurization processes, and certain non-food and non-pesticidal agricultural applications such as nitrogen fixation.

Microorganisms used in the production of a chemical end product are subject to TSCA if the end product is a chemical substance used for a purpose other than as a food, food additive, drug, cosmetic, or medical device.

Under FIFRA, uses of pesticides which would be regulated include pesticides used on agricultural plants, grain fumigants, and insect sprays.

The definition of environmental release will determine, to a large extent, the scope of coverage of EPA regulatory policies.

The Meeting

Format

Defining environmental release for TSCA and FIFRA purposes raises new and complex technical issues. In order to tap a broad spectrum of expertise in its effort to develop a workable definition of "release," EPA assembled a group of recognized technical experts as a subcommittee of the EPA-based expert advisory committee, the Biotechnology Science Advisory Committee (BSAC). This subcommittee, the Subcommittee on Definition of Release to the Environment, met in public session on December 11-12, 1986, in Crystal City, VA. The public was given opportunity to comment.

Appendix I contains a roster of the subcommittee. Appendix II contains the minutes of the meeting.

On the first day of the meeting the issues and EPA's goals were explained. Specific comments and observations were solicited from each participant from the perspective of his expertise and research experience. The group was then asked to "brainstorm" and attempt to suggest as many approaches as possible. The subcommittee was asked at this point not to attempt to judge the acceptability or credibility of the approach. The subcommittee would comment on the advantages and disadvantages of the approaches later in the meeting.

After several approaches were suggested, subcommittee members were assigned to smaller groups to write language for the proposed approaches.

The subcommittee was told that from the Agency's perspective the most important characteristics of a "good" definition of "release" were:

- Flexibility to adapt to advances in the technology. A "good" definition is one that can be applied to future applications not currently envisaged.

- Ease and simplicity of interpretation. It is advantageous to both the Agency and industry to be able to determine readily and easily whether the use of a particular product constitutes an environmental release. Ideally, both the Agency and industry should be able to make the determination using existing data. A definition of environmental release that would require testing in order to determine whether the product would fall within the definition would be undesirable.

Examples of possible approaches were also offered. Included were the following examples:

- a general definition with specific guidance as to the types of procedures that could be included or excluded from the definition;

- different definitions for different categories of uses (e.g., one definition for waste degradation uses, another for agricultural uses);

- a definition describing containment rather than release; any use which is not contained is considered a release.

It was emphasized that the subcommittee was not limited to these suggestions, but was encouraged to develop a variety of approaches.

Information thought to be relevant to the discussion was supplied to the subcommittee. Brief descriptions of the articles are provided in Appendix III.

Objectives

The Agency hoped the subcommittee could provide:

- several approaches to defining environmental release;

- discussion of the scientific and technical justifications, advantages, disadvantages, limitations, and implications of the approaches;

- consideration of the approaches in terms of the population dynamics of released organisms.

Approaches to Defining Release

Seven approaches for defining release into the environment were suggested by the subcommittee. These seven approaches suggest a definition of release could be based on:

1. the *numbers* of organisms released to the environment;

2. *use;*

3. a consideration of methods of *controlling* organisms;

4. a definition of *containment;*

5. consideration of *risk;*

6. categories of *organisms;* and

7. a combination of the *containment* based and *organism* based approaches.

Approach Based on Numbers of Organisms Released to the Environment

This approach proposes that a number of organisms released to the environment would trigger EPA review. For regulatory purposes, a release would be greater than 10^x of a particular organism, where 10^x is the amount released per day from a greenhouse built and operated according to specifications for a "good greenhouse practices greenhouse" (GGHP).

The standard for a GGHP greenhouse should be a "contained" greenhouse built according to a design and operated under conditions specified by EPA.

Procedures conducted in the stages of research and development which precede the field trial, e.g., laboratory experiments and growth chamber procedures, should be conducted using good standard practices and procedures. Risk evaluation would be performed at each step in development as part of good practices. As activities are scaled up, more organisms will probably be released from the facility, because more organisms will be used in larger scale procedures. However, appropriate practices would be followed and there should be fewer organisms released in these developmental stages than in the field test. The requirement for good laboratory practices and procedures was added for the logic of the approach; how could a certain number of organisms be defined as release if that

number had already been "dumped" in the environment in prior development steps because of poor technique.

Exemptions from EPA regulation would be recognized. Suggested examples of exempt categories for small field trials were:

". . . indigenous non-pathogenic organisms and organisms derived from simple deletions, single base changes, rearrangements and amplifications within a single genome. . . ."

Since the trigger for review is the number of organisms released from a GGHP greenhouse per day, a small field trial involving fewer organisms than would be released from a GGHP greenhouse would not be considered a release regulable by EPA.

Comments by Meeting Participants. The following observations were made concerning this approach:

- It is difficult to choose a number of organisms which would encompass all possible applications. For example, the numbers of organisms used in a field test in one industry might be orders of magnitude greater than those used in another industry.
- Organisms dispersed from a facility such as a greenhouse in the course of operations may be released over time through a number of mechanisms; dispersion through filters, dispersion through water runoff, dispersion on employees' clothing, etc. In the field test, all of the organisms to be released are applied at the same time in a small area. The dose would probably be larger in the field test than through inadvertent dispersion from a facility such as a greenhouse. In a field test, the total dose would also most likely be specifically directed at a "target" host or receptive ecosystem.
- What constitutes a GGHP greenhouse would have to be determined.
- This approach has a certain appeal from a regulatory standpoint because a number value can be quantified and is objective.
- This approach might be combined with use categories.
- This approach should be considered from the point of public perception; the public might consider the trigger number to be a very large number of organisms.

Use-Based Approach (Pilot-Scale)

This approach was initiated as an attempt to base a definition of release on "use." However, it is difficult to develop a definition based on use because of all the applications both known and unknown of microorganisms in biotechnology. However, all research and development proceeds through several stages: from laboratory (bench scale), to prototype (pilot scale), to product, to commercial use. Since the NIH Guidelines for Research Involving Recombinant DNA Molecules cover laboratory testing, the move to pilot scale testing was suggested as the trigger for EPA review.

Pilot scale is defined as a limited discharge in an isolated geographic area. Pilot scale might include:

- In agriculture, a greenhouse, if a greenhouse were defined as pilot scale;
- In metal mining or ore leaching, a thousand tons of rock a day;
- In the oil industry, 25,000 to 50,000 barrels per day;
- In the pesticide business, 10 to 100 liters.

Under this approach, EPA might also develop a list of exemptions.

Comments by Meeting Participants. The following observations were made concerning this approach.

- A definition of "pilot scale" would have to be developed.
- Pilot scale would vary with the industry. In some applications such as mineral mining and oil recovery pilot scale sounds rather large.

Control-Methods Approach

This approach, which is based on "control methods," employs a point scheme. Points would be assigned to biological and physical control measures. Examples of control measures include: certain features of the organism; various physical barriers; or remedial activities. Through addition, subtraction, or multiplication the points assigned to each control measure would be summed to obtain a point total. EPA would select the point total which would trigger review.

Examples of control measures include:

Biological:

- Viability (hardiness)
- Suicide Gene
- Environmental Requirements (temperature, pH)
- Quantity of organisms released

Physical:

- Barriers (walls, filters, etc.)
- Remedial measures (e.g., chemicals or temperatures).

EPA would have to develop appropriate criteria and assign points to the criteria. It would also have to determine the number of points which would trigger a release. One advantage of this scheme is that EPA could adjust the trigger point in light of experience. Another advantage is that points could be assigned for applications not currently envisaged.

The system permits companies to determine the approach they would take to obtain the points. Physical barriers or remedial activities or containment could be emphasized if the company wished to pursue such a pathway; alternatively the company might prefer to focus on biological containment such as developing a "suicide gene" system. This point scheme approach would offer industry greater flexibility in research and development design: Industry could determine how it wishes to obtain the necessary point value since the scheme allows industry to "trade-off" among factors.

Comments by Meeting Participants. The following observations were made concerning this approach.

- EPA would have to choose the control methods and determine the point values to be assigned to each method.

- The point system would have to be based on objective measurable values as opposed to subjective criteria. This is particularly true in choosing biological containment criteria since such criteria are more subjective. However, under this scheme the number of points assigned to any criterion could reflect the degree of confidence associated with the control method.

- The trigger point for EPA review could be a single number or could vary with the use or industry.

- This approach requires further development but from a scientific point of view has certain inherent attractions; the criteria are quantifiable and the scheme offers flexibility to both industry and the Agency.

Release as Opposite of Containment

This approach, which is based on a concept of containment, specifies that EPA notification and review will occur prior to conducting any testing or procedure in an "unrestricted environment" regardless of the size of the test or procedure.

The following language was offered for discussion:

"For organisms defined as pathogens, EPA notification and review will precede growth chamber, growth room and/or greenhouse tests, and such growth facilities shall include a level of containment from which no greater than 10^x organisms per day are emitted.

"For intra-generic and non-pathogenic organisms, EPA notification and review will [occur] prior to conducting any level of evaluation in an unrestricted environment regardless of the size.

"This includes tests on biologically contained organisms until the efficacy of the genetic containment has been demonstrated to be acceptable."

The term "unrestricted environment" is used rather than the term "field" because some uses would not be in a "field" type of application.

This approach would not recognize any difference between "biologically contained" and organisms not constructed to be "biologically contained" since the efficacy of such containment could not be evaluated prior to actual testing in the field.

Two triggers are included in the definition: (1) use of pathogens, and (2) use of nonpathogenic organisms constructed from source organisms from different genera; this is consistent with EPA's current policy.

A list of exemptions could be developed for small scale field tests.

Comments by Meeting Participants. The following observations were made concerning this approach.

- The term "unrestricted environment" would have to be defined. It might prove difficult to define an "unrestricted environment."

Risk Based Approach

This approach is based on an evaluation of risk as a trigger for EPA review. Under the risk based approach, low, medium and high risk ratings would be applied to criteria in three categories: (1) biological factors; (2) applications/use factors; and (3) environmental factors.

Examples of biological factors offered include: number of organisms released (this would vary with the type of organism); genetic stability; pathogenicity; survivability; controllability; host range; and indigenous versus exotic origin.

Examples of the application/use criteria include: manufacturing; biorational (biocontrol); oil recovery; agricultural (pesticide or fertilizer); and metal reclamation.

Examples of environmental criteria include: laboratory; greenhouse; field; commercial production facility; fresh water systems; and marine water systems.

The subcommittee did not have sufficient time to develop all the criteria they thought would be appropriate for this approach. They did, however, rate some criteria and classify them in three categories. Items in category C correlate with risk, are relatively easy to measure and are objective; items in category I are intermediate in correlating with risk, ease of measurement and objectiveness; items in category P correlate poorly with risk, are not easily measured, and are not objective.

Of the biological factors, pathogenicity and controllability fall into category C; genetic stability, survivability, ability to monitor, competitiveness with nontarget organisms fall into category I; host range and bioproducts released fall into category P.

All of the application/use factors rated by the group fall into category C. These include: aerosol, liquid, above ground, below ground, enclosed/open, growing, nongrowing, numbers/volume (concentration).

The three environmental factors rated by the group, site suitability, species, hydrology, fall into category I.

Comments by Meeting Participants. The following observations were made concerning this approach.

- The concept of a point scheme could be applied to this approach. The criteria used in the point scheme would be different, however, from those used in a control-based approach.

It is difficult to assign numerical values to criteria listed in a risk approach, such as pathogenicity and survivability, many of which are subjective and context dependent.

- The approach is data intensive for both industry and EPA.

- The amount of testing necessary for industry to determine whether a procedure is a release would be high. Standardized tests would probably be required to permit comparison between companies and to offer industry some solid criteria on which to determine the status of a procedure.

- Who would evaluate the criteria in order to know a procedure would be a release? Would the company perform such a review itself? Would EPA be involved?

• The approach requires a risk assessment be performed in order to trigger risk assessment by EPA.

• This approach does not offer a simple definition readily usable by EPA.

Organism-Based Approach

This approach is an attempt to utilize categories of organisms to define release. Under this approach, review would be required for certain situations; for example, *all* pathogens, organisms being placed in new niche, or for new use of an organism.

For some situations, review would not be necessary. Examples suggested for exemption were: indigenous saprophytes reintroduced in their niche; organisms incapable of replicating and transferring genes; or when the product is cell or DNA free. Evidence would have to be provided for evaluation prior to inclusion in the exempt category. Indigenous saprophytes reintroduced into their niche should be shown to be not able to transfer genetic material before they could be considered exempt.

EPA would be notified whenever experiments begin in the laboratory if the ultimate intent is release to the environment.

Tables of organisms could be developed based on use.

Comments by Meeting Participants. The following comments were made on this approach.

• Factors such as controllability are important in determining whether an organism has been released but these factors are ignored by this approach.

• This approach is based on taxonomy which is not at this time based on evolutionary relatedness.

• What constitutes a "new" niche for the organism would have to be defined.

• With this approach, in time, a table of exempt organisms with accepted uses and applications could be developed.

Containment/Organism Based Approach

This approach combines a containment approach with an organism based approach.

A chart would be constructed where containment levels would be shown on the horizontal axis, while categories of organisms would be on the vertical axis. The chart would show when review would be triggered as a function of level of containment and organism categories. For example, review might be triggered for one category of organism at a certain containment level; for another category of organism, review might be triggered at a different containment level.

Five levels of containment were envisaged. The highest level of containment would prevent release of most of the organisms. The next level of containment would be similar to the highest level of containment except that the performance standards would be lower. The third category would be a "shielded" type of containment; i.e., a setting buffered from the natural environment by some type of physical separation from niches where the organism might be able to grow and survive. The traditional greenhouse might fall in this category. The fourth level would be a setting, such as a field plot, where the release can be mitigated by chemical or physical means. The fifth level would be a setting, such as a field plot, limited by some restriction such as acreage.

Included within these levels of containment would be: growth chamber, growth room, contained greenhouse, greenhouse, and pilot plant.

Several general categories of organisms were envisaged. Examples of the types of categories include: human pathogens, animal pathogens, plant saprophytes, etc. Within these categories, subcategories could be created.

Comments by Meeting Participants. The following comments were made concerning this approach.

• The major problems with such an approach are: can the levels of containment be clearly defined; can the levels be clearly defined for all applications, particularly those which do not involve testing in the agricultural field plot; can categories of organisms be defined which do not have so many exceptions the categories are useless.

• This scheme could become quite complex if the number of categories of organisms increased.

• The advantages of this approach are: the trigger is clearly defined; since the categories of organisms can be moved with respect to containment, the scheme is flexible.

• This approach contains an element of hazard evaluation; hazard evaluation is implicit in the development of categories of organisms.

• This approach is data intensive for EPA.

Discussion

Several approaches to defining "release" to the environment when microorganisms are used to perform certain tasks were suggested by the subcommittee. These approaches were based on: (1) the number of organisms released to the environment; (2) use; (3) control methods; (4) containment; (5) risk; (6) categories of organisms; and (7) a combination of the containment based and organism based approaches.

The EPA will evaluate the suitability of these approaches and any approaches of it may develop against several criteria.

The Subcommittee on Definition of Release suggested several criteria against which to measure the suitability of the approaches to defining release. These are: clearly definable review trigger; consistent with current information; easy to understand and implement; credibility; encouraging of information gathering; numbers of false positives and false negatives; appropriateness to TSCA and FIFRA; flexibility to incorporate new information and new uses; and flexibility to accommodate exclusions.

The several approaches to defining release developed by the subcommittee and any other approaches EPA may develop will also be analyzed by the Agency for: scientific credibility; legal, policy, and economic implications, resource implications for both the Agency and the industry, ease of implementation, and technical feasibility.

In addition, the Agency will evaluate the approaches in light of how they mesh with recognized procedures for assessing risk. Briefly, a risk assessment in the EPA con-

sists of an evaluation of the two components of risk; exposure and hazard. In the exposure assessment portion of the review, the distribution of the substance/product is described. Exposure assessment emphasizes the fate of the substance/product in the environment and the cumulative amounts which might reach other organisms. In the hazard assessment portion of the review, the potential adverse impacts of the substance/product on organisms and processes are identified, then quantified. These two assessments are then combined to characterize risk.

Risk management, the step beyond risk assessment, involves the determination of what, if any, regulatory measures are appropriate to eliminate or mitigate the identified risks. Under both TSCA and FIFRA, risk management seeks to reduce the identified risks to "reasonable" levels through controls on manufacture, use and disposal, and through mitigation, monitoring and other activities. The determination of whether a given risk is unreasonable involves the comparing of risks and costs to benefits and other values. Risk assessment and risk management are interactive and adaptive.

Because of the way risk assessments are performed within the Agency and because the definition of release will serve as a "trigger" for review, the best definition of "release" would most likely be based on exposure rather than on risk. A definition based on risk would require that two parameters, exposure and hazard, be evaluated and would most probably require testing in order to determine whether the procedure would constitute a release reviewable by EPA.

The results of this analysis of the approaches to defining release will be published for public comment as part of the Agency s rulemaking process. After consideration of public comments and regulatory and policy issues, EPA will issue final rules incorporating a definition of "release" to the environment.

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9. [FIFRA] Federal Insecticide, Fungicide and Rodenticide Act, 25 June 1947, and as amended U.S. Code Title 7, Section 136-136y.

10. [TSCA] Toxic Substances Control Act. 11 October 1976 and as amended U.S. Code, Title 15, Sections 2610-2629.

Appendix 1

Roster for the BSAC Subcommittee on Environmental Release

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Appendix 2

Minutes Of The Meeting[1]

The Subcommittee on Definition of Release to the Environment of the Biotechnology Science Advisory Committee (BSAC) was convened at 9:00 a.m. on December 11-12, 1986, in room 1112 in Crystal Mall 2, Jefferson Davis Highway, Crystal City, VA 22202. The meeting was open to the public. Dr. James Tiedje was Chair. A subcommittee roster is attached to the report. The following people were present for all or part of the meeting:

Subcommittee Members:
Lewis Albright
William Dowler
W.R. Finnerty
Charles Hagedorn
Eric Krabbe
David Lincoln
George Pierce
Douglas Rouse
Max Summers
James Tiedje
Paul Wichlacz
Elizabeth Milewski (Executive Secretary)

Other EPA Staff:
Frederick Betz
Anne Hollander
Jane Rissler

Other:
Marianne Anderson, Hurton and Williams
E. J. Baier, Occupational Safety and Health Administration
Jodi Bakst, Environmental Protection Agency
Don Beers, McCutchen and Doyle
Irene Brandt, Eli Lilly
Peter Carlson, Crop Genetics
Kate Devine, Environmental Protection Agency
Janet Dorigan, Department of Energy
Charles Eby, Monsanto
Debra Edwards, Environmental Protection Agency
Gerald Flak, Environmental Protection Agency
Bob Frederick, Environmental Protection Agency
Elaine Friehele, Versar, Inc.
Rebecca Goldberg, Environmental Defense Fund
Alan Goldhammer, IBA
Ron Grandson, Pesticide and Toxic Chemical News
G.A. Hapka, DuPont
W.F. Harris, National Science Foundation
William J. Hazel, Environmental Protection Agency
Theresa Hibbens, Inside EPA
Daniel Jones, U.S. Department of Agriculture
Rosamund Katz, Government Accounting Office
Jan Kurtz, Environmental Protection Agency
Gregory Macek, Environmental Protection Agency
James K. Marsh, Jellinek, Schwartz and Connolly
Greg McCaffery, BNA
Henry Miller, Food and Drug Administration
Jack Moore, Environmental Protection Agency
P. Nishimi, Office of Technology Assessment
Angela Nugent, Environmental Protection Agency
Michelle R. Owings, Burditt, Bowles and Ratzius
Anne Plant, Bureau of Standards
Steven W. Rauch, Merck and Co. Inc.
Ed Raleigh, DuPont
Christine Riley, Beveridge and Diamond
Shirley Ross, Environmental Protection Agency
Philip Sayre, Food and Drug Administration
Warren Schultz, Department of Defense
Mark Segal, Environmental Protection Agency
Bill Schneider, Environmental Protection Agency
Janet Shoemaker, American Society of Microbiology
Roy Sjoblad, Environmental Protection Agency
Mike Swit, Burditt, Bowles and Radzius
William Szkrybalo, Pharmaceutical Manufacturers Association
Greg Thies, Environmental Protection Agency
Zig Vaitusis, Environmental Protection Agency

Call to Order and Introductory Remarks

At 9:00 a.m. on December 11, 1986, Dr. Jane Rissler of the Office of Toxic Substances (OTS) of the U.S. Environmental Protection Agency (EPA) welcomed the participants and observers to the December 11-12, 1986, meeting of the Subcommittee on Definition of Release to the Environment of the EPA Biotechnology Science Advisory Committee (BSAC). She thanked them for participating in this effort to develop approaches to defining environmental release.

Dr. Rissler introduced two other EPA representatives: Dr. Elizabeth Milewski of the Office of Pesticides and Toxic Substances (OPTS), the Executive Secretary of the Subcommittee; and Mr. Frederick Betz of the Office of Pesticide Programs (OPP). Dr. Rissler said she and Mr. Betz were serving as Agency resource persons. Dr. Rissler said Ms. Anne Hollander of OTS would serve as recorder.

Dr. John A. Moore, EPA Assistant Administrator for Pesticides and Toxic Substances, thanked the subcommittee members for their willingness to participate in grappling with this difficult issue. He said the public is keenly aware of this technology and has tremendous curiosity about it. At this time, the challenge for those involved with biotechnology is to develop a publicly acceptable process to ensure the transition from a curiosity to a "routine" technology.

[1]The Subcommittee is advisory to the BSAC which is advisory to the EPA. Its recommendations should not be considered final or accepted.

Dr. Rissler introduced the Chair of the BSAC Subcommittee on Definition of Release to the Environment, Dr. James Tiedje of Michigan State University.

Dr. Tiedje said the goal of the meeting was to develop several approaches for defining release to the environment for microorganisms used to perform certain tasks. He said the Subcommittee would first "brainstorm" to suggest approaches. The Subcommittee was asked at this point not to judge the credibility or acceptability of an approach. Subcommittee members would later be assigned to smaller groups to write language for these options. These small groups as well as the Subcommittee itself would explain the advantages and disadvantages of each approach.

Dr. Tiedje pointed out that Dr. Milewski had provided a summary of the December 5, 1986, meeting of the National Institutes of Health (NIH) Working Group on Definitions of the NIH Recombinant DNA Advisory Committee (RAC). He asked Dr. Milewski to report briefly on that meeting.

Dr. Milewski said the NIH working group is considering the issue of environmental release. The NIH group felt they should address the NIH's sphere of responsibility, i.e., research, rather than the needs of other federal agencies. The December 5, 1986, discussion was targeted to what the NIH working group called "planned releases." They suggested appendices specifying criteria for planned releases involving microorganisms, plants, animals and vaccines be written for incorporation in the NIH guidelines. If a proposed planned release met the criteria specified in the appropriate appendix, the planned release would be subject to a lower level of scrutiny of review than those that did not meet the criteria.

An observer at the December 11-12 EPA meeting, Dr. Henry Miller of the Food and Drug Administration said the NIH working group attempted to "carve out" large categories of exemptions from the NIH Guidelines. Dr. Milewski said her report on the December 5, 1986, NIH meeting describes the categories suggested for exemption. These include: single base changes, rearrangement and amplifications within a single genome. She pointed out that under EPA's biotechnology policy, organisms constructed using these categories of manipulations are generally not subject to the higher scrutiny level of review before being tested in the field.

Dr. Rissler and Mr. Betz then described EPA's goals relating to regulation of environmental release of microorganisms covered by EPA's policies. Dr. Rissler said EPA must develop a definition which permits both the Agency and industry to determine whether a particular use of a microbial product constitutes a release to the environment. She said a definition of environmental release is central to implementation of EPA's regulatory scheme for biotechnology products under the Toxic Substances Control Act (TSCA) and the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). The definition will determine, to a great extent, the scope of these policies.

Dr. Rissler said the approaches for defining release suggested by the subcommittee would be used in the process to implement these two statutes as they apply to biotechnology. The Agency will evaluate the approaches suggested by the subcommittee and any options it may develop on its own, in a regulatory and policy context. The results of this analysis will be published for public comment in the *Federal Register.* Public comment and other considerations will be evaluated in the writing of final rules to fully implement the biotechnology policy.

Dr. Rissler explained that EPA will regulate biotechnology products under TSCA primarily in four ways. One of these, the review of commercial uses of "new" microorganisms under the section 5 premanufacture notice (PMN) process, does not depend on a definition of release to the environment, and is already in effect.

However, the other three processes are tied to the concept of environmental release and therefore necessitate a workable definition of release. These three provisions are:

- Section 5 rule: the Agency will amend this rule so that environmental releases in the course of "research and development" will not be exempt from PMN review. EPA believes that living organisms are not "small quantities for research and development" because of the ability of living organisms to persist, multiply and spread.

- Section 5(a)(2): EPA will issue a significant new use rule (SNUR) which will cover certain microorganisms that are pathogens or organisms deliberately altered to contain DNA from pathogens.

- Section 8(a): EPA will promulgate a regulation in order to require reporting of minimal basic information. This provision will provide a mechanism of monitoring the industry and ensuring that there will be an increasing base of information to assess and modify regulatory decisions.

Mr. Betz said a definition of release is necessary to fully implement the FIFRA environmental use permit provisions as they relate to the requirements for releases of certain products of biotechnology. Because all large scale uses of pesticides are overseen under FIFRA, the concept of release is not as critical to the regulatory process once a pesticide product has moved beyond small scale trials.

Dr. Rissler then described how TSCA applies to certain products of biotechnology. Under TSCA all microorganisms produced for environmental, industrial, commercial or consumer uses are potentially regulable with certain exceptions: i.e., microorganisms used as food, food additives, drugs, cosmetics, medical devices and pesticides, and microorganisms used to produce food, food additives, drugs, cosmetics and medical devices.

Examples of products subject to TSCA include microorganisms used to convert biomass to energy, degrade pollutants, treat sewage, enhance oil recovery, extract and recover metal, desulfurize fossil fuel, and increase agricultural productivity. Microorganisms used to produce chemical end products are also subject to TSCA.

Ms. Hollander described how biotechnology products will be evaluated by OTS. There are two levels of review for biotechnology products. One level requires more data and provides greater scrutiny; pathogens and organisms constructed from source organisms from different tax-

onomic genera are in this group. Fewer data need be submitted for the other level.

Mr. Betz explained how FIFRA has been implemented to regulate pesticide products of biotechnology. Under FIFRA, OPP conducts a premarket review and risk assessment of pesticide products. These products must be registered before they can be marketed. Under FIFRA, data are required on human health and ecological effects and environmental fate of pesticide products; responsibility for developing that data rests with industry. OPP evaluates the data and may register the product, provided the data support the conclusion that use of the product will not pose an unreasonable risk to humans or the environment. OPP has reviewed a number of microbial pesticides over the years; currently some 14 products are registered for use.

The types of products OPP would review include agricultural products for crops, forestry and homeowner pesticides. Some pesticides might be applied in buildings, but not necessarily in a contained situation; e.g., grain fumigants, products used in other agricultural buildings, and crack and crevice cockroach spray.

Mr. Betz said two levels of scrutiny exist for notifications under the FIFRA experimental use provisions for biotechnology products to be tested in small scale field tests; an abbreviated 30 day review requiring a minimal amount of information, and a 90 day review requiring more information. If the finding in the 30 day review is that there are no significant risks, testing may proceed. If additional information is required, the application could be given a Level 2 review or if the submitter so requests, an experimental use permit review.

Mr. Betz provided a flow chart (Figure 1) of the development process for microbial pesticides. Generally development proceeds from the laboratory, to growth chamber or greenhouse, to small scale field testing, to large scale field testing, and finally to marketing. Currently, OPP considers laboratory and greenhouse testing situations to be contained. The other three situations are considered release. At the present time, the concept of contained is central to OPP policies as they relate to notifications for small scale use. The Agency currently considers release to the environment to be any procedure that occurs outside of a contained situation as defined in the June 26, 1986, *Federal Register* policy statement on biotechnology.

Mr. Betz said OPP would wish to adopt an approach to defining release compatible with previous biotechnology policy statements.

Brainstorming Session

Dr. Tiedje then asked the members of the subcommittee to comment from their expertise and experience on the subcommittee mandate. He said he is a microbial ecologist interested in how organisms compete and succeed in the environment.

Dr. Charles Hagedorn of Virginia Polytechnic Institute said he is a microbial ecologist interested in the biology of nitrogen fixation and biorational pesticides. He suggested the concept of "niche restriction" might be one approach to developing a definition of release; an organism which will die out following application might be considered "restricted."

Dr. Hagedorn said another approach to defining release to the environment might be to specify a number of organisms as the trigger for EPA review. How the "trigger" number is chosen is an important issue if this approach is adopted.

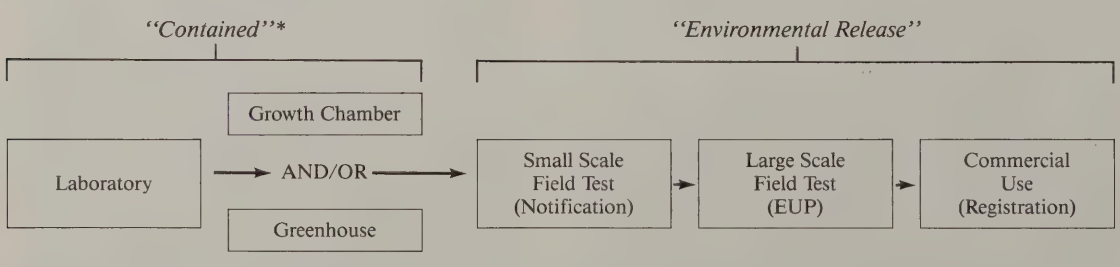
Dr. Louis Albright of Cornell University said his expertise is in environmental control in agricultural buildings, particularly greenhouses. Referring to the "numbers" approach mentioned by Dr. Hagedorn, he said it would be difficult to reduce the number of microorganisms released from a greenhouse per day to the number of microorganisms released from a BL1 laboratory per day.

Dr. David Lincoln of the company, CH2M Hill, said his expertise is in modeling the release of microorganisms from operating facilities. He suggested the definition adopted by EPA ought to be "information encouraging."

FIGURE 1

Environmental Release: Current FIFRA Perspective

Development Path for "typical" genetically altered microbial pesticides—under the existing regulations, policies, and definitions



*Laboratories, growth chambers and greenhouses can be, but are not necessarily, "contained" facilities. Appropriate levels of containment can be assigned to specific microorganisms based on risk.

Mr. Erik Krabbe of the consulting firm, Bioengineering, said his expertise is in the fermentation industry. He added that because of his background he tended to think in terms of containment.

Dr. Max Summers of Texas A & M University said his expertise is in insect virology, molecular biology and genetically engineered viral and bacterial pathogens of insects. He said he would have a problem with a definition of release based on containment. To his mind, if one organism were to escape from a containment facility, the organism is not contained, even though it may be restricted. He thought the definition of release should be dictated by the nature of the organism, the nature of the engineered gene, the purpose for which the product was developed, and how it is introduced into the environment.

Dr. George Pierce of Batelle Memorial Institute said his area of expertise is in the genetics and physiology of organisms, primarily the pseudomonads, which degrade toxic pollutants. He said in his experience containment does not imply lack of release. Containment is a means of engineering additional methods to control risk.

Dr. Paul Wichlacz of the Idaho National Engineering Laboratory said his expertise is in the ecology and physiology of organisms used in mining and extraction of metals from ore. He thought containment depended on the situation. In the mining industry, microorganisms are sprayed onto millions of tons of ores. Containment for that scale of operation would be virtually impossible. However, the organisms used in metal extraction survive and thrive in a very restricting environment. They have to tolerate grams per liter of metals and very low pH (1.5). The source organisms for genetically modified organisms used in ore leaching would most likely be from that environment as would the genes used to modify them. He said the concept of "niche restriction" is thus attractive to him.

Dr. Douglas Rouse of the University of Wisconsin said his expertise is in epidemiology, specifically modeling of plant disease progression. He thought the concept of niche restriction would involve defining release in terms of where an organism can go after it leaves containment and whether it can find a niche in which to survive and reproduce. An issue associated with this concept is whether survival and multiplication represents a risk to the niche. He thought, however, the subcommittee would have some difficulty including this consideration in a definition of release.

Dr. William Finnerty of the University of Georgia said his expertise is in the microbial physiology of microorganisms used in oil recovery. He said most consideration is directed towards microorganisms; he questioned whether some concern might not be appropriately directed towards the promiscuity of genes. The genetic potential of the environment has not been adequately evaluated and the impact of introduced genes on the environment is unknown. For example, if it takes 50 years for a single gene from a single organism to distribute through a microbial population, how long would it take for the same gene contained in a large number of introduced bacteria to distribute?

Dr. William Dowler of the USDA Agricultural Research Service in Beltsville, MD, said he is a plant pathologist with a background in fungal physiology. His laboratory's mission is to develop fungal biocontrol agents for weeds. He felt risk should be part of the definition of release. If the released organism is a pathogen with a low active dose, one organism released may be too many. For other organisms a few hundred thousand probably would make no difference.

Dr. Lincoln said a definition of release may be looked at in two ways: (1) as a scientific definition which would include the concepts of risk and hazard; and (2) as a trigger for review. He asked whether risk could be used as an approach to triggering review. Dr. Rissler said EPA will use a definition of release as a trigger for review; the review itself is a risk assessment. It is difficult to see how a definition which depends on a risk assessment would be implemented. Definitions which do not depend on a risk assessment are thus preferable. However, the subcommittee has been asked to develop several different approaches to defining release and is not precluded from developing an option based on risk.

Ms. Hollander explained that the Agency would perform a risk assessment on a case-by-case basis by evaluating two components of risk, hazard and exposure. Two groups of EPA specialists perform a risk assessment; one group examines hazard, the other exposure. These two evaluations are then combined into an analysis of risk. After the risk assessment is complete, a risk/benefit evaluation may be performed. Finally a regulatory decision is made by the Agency. In general, a definition of release based on exposure would be easier to implement since it is based on one parameter; a definition based on risk would be more complex and difficult to implement because it depends on both exposure and hazard.

Ms. Hollander pointed out that a distinction between review and regulation should be drawn. The Agency could review a product and the determination made that the product would not be regulated.

Dr. Tiedje asked the subcommittee to list criteria relevant to a definition of release to the environment. He listed the numbers of organisms released as relevant. How the organism is used is also relevant. These two concepts could be combined; for example, different numbers of organisms might serve as triggers in different use situations.

Dr. Summers said how the organism is introduced to the environment is relevant.

Dr. Hagedorn said "niche restriction," the ability to survive and grow in a particular setting, is also relevant. Niche restriction would be affected by where the organism was released. Dr. Hagedorn added physical and biological containment to the list of pertinent considerations.

Dr. Friehele of Versar, Inc. suggested the presence or absence of "receptors," i.e., target niches, are important. In a risk assessment, transport pathways which might disperse the organism to receptors would be evaluated.

Dr. Finnerty suggested "genetic potential," the ability of a gene to disperse in the environment, might be considered.

Dr. Rissler suggested "ability to control after release" be added to the list. Control could be effected by mitigation or characteristics of the organism.

Dr. Wichlacz said a definition of release would draw a line somewhere along the development continuum which flows from laboratory testing to commercial production. Accidental release is an issue distinct from testing. Dr. Tiedje thought a definition of release should apply to accidental release or deliberate dumping as well as to planned releases.

Dr. Pierce thought "efficacy" might also be a pertinent criterion which should be added to the list.

Dr. Rouse suggested the type of organism might be a pertinent criterion, as would the ability to monitor the organism.

Dr. Pierce suggested the number of organisms released is important and is an "amplitude factor"; larger numbers increase the probability of impact if the organism presents a hazard.

Dr. Rouse suggested the relevant considerations offered by the subcommittee could be grouped into three broad categories as follows:

Category 1—Number of Microbes Released
Number of organisms
Physical containment
Definition that draws line somewhere along research and development continuum

Category 2—Exposure
Use
How it's introduced
Biological containment
Niche restriction
Ability to survive and grow
Genetic impact/potential
Ability to control after release
Where it's released
Definition that draws line somewhere along continuum
High probability of release
Type of organism
Ability to monitor
Receptors (organisms that would be affected)

Category 3—Hazard
Relates to risk and benefit consequences
Use
Genetic impact/potential
Definition that draws line somewhere along continuum
Acceptable standards
Efficacy

Dr. Rouse noted that many of the concepts fall under exposure. This underscores the importance of exposure to the issue of release.

Dr. Wichlacz thought a definition for release could be based on risk and encompass the number of organisms released, exposure and hazard.

Dr. Mark Segal of OTS said he was puzzled by the suggestion to employ risk in a definition of release meant to trigger regulatory review. He found it difficult to see how a definition based on risk could be used as a trigger for review. There is currently a general consensus that biotechnology products must be evaluated for risk on a case-by-case basis. How this evaluation would be performed before review is triggered is not evident.

Dr. Summers thought the phrase "adequate evidence of biological and/or physical control . . ." used by the NIH working group suggested consideration of risk.

Dr. Tiedje said the phrase "adequate evidence of . . . control" would require companies to perform tests before they could determine whether a procedure involved a release to the environment. He questioned whether testing should be required for a determination of release.

Dr. Rissler said this type of trigger appears to require a risk assessment. Logically, EPA could not perform a risk assessment to determine whether a procedure is a release which must be reviewed for risk. The definition of release should trigger a risk assessment rather than requiring that one be done to determine whether a risk assessment should be performed. Another stumbling block is the term "adequate evidence." What is adequate evidence and who determines it is adequate?

The subcommittee then suggested a number of approaches to defining release. The list includes:

"1. number of organisms released
2. use
3. control measures
4. definition of containment
5. risk; and
6. type of organism."

Dr. Tiedje assigned subcommittee members and staff to 2 or 3 person groups to write language for each of these suggested options. Drs. Rissler and Tiedje would write language for the option based on the number of organisms released. Drs. Wichlacz and Summers were assigned the task of writing language for the use-based option. Drs. Lincoln and Milewski were assigned the control based option. Drs. Albright and Hagedorn were assigned the option based on a definition of containment. Drs. Dowler, Finnerty and Mr. Krabbe were assigned the risk-based option. Drs. Rouse and Pierce were assigned the organism-based option.

Description of Approaches

Numbers of Organisms

Dr. Tiedje explained the language he had developed with Dr. Rissler for a definition based on numbers of organisms released. Dr. Tiedje said the key language is that:

"releases greater than 10^x of a particular organism per day, which is the amount released from a greenhouse built and operated according to x specifications (Good Greenhouse Practices)"

constitute a release reviewable by EPA.

Dr. Tiedje said this approach is based on the assumption research would begin in the laboratory under appropriate laboratory practices and procedures. Information will be generated in the laboratory which could be used to evaluate risk in subsequent steps in the continuum from laboratory to field application. Each step in the continuum will probably result in the release of larger

numbers of organisms, but data would have been evaluated in prior steps and appropriate practices would be followed to restrict the organism.

The requirement for appropriate laboratory practices and procedures had been added for the logic of the approach; how could a certain number of organisms be "defined" as release if that number had already been dumped in the environment in prior development steps because of poor technique.

Since the trigger for review is the number of organisms released from a GGHP greenhouse per day, under this approach, a small field trial involving fewer organisms than would be released from a "good greenhouse practices" (GGHP) greenhouse would not be considered a release.

Dr. Tiedje said an exemption category could be developed in this approach. Examples of the types of organisms included in the exempt category would be releases involving indigenous non-pathogenic organisms and organisms derived from simple deletions, single base changes, rearrangements, and amplification within a single genome. Investigators would have to meet good laboratory, greenhouse, or large scale practices if the organisms were in the exempt category. Procedures utilizing these organisms would not be reviewed prior to large scale field testing.

Dr. Summers pointed out that the requirement to meet appropriate laboratory practices and procedures prior to field testing would extend EPA purview into the laboratory.

Dr. Rissler said the important point in this approach is the number of organisms which would trigger review; the statement on good practice in the laboratory would not be a trigger for review and would not extend EPA's purview.

Dr. Dowler asked how a GGHP greenhouse would be defined. Dr. Tiedje replied that the standard GGHP greenhouse would be built according to specified designs and operated under conditions determined by EPA. The number of organisms released from a GGHP greenhouse would be higher than the number released from a BL1 laboratory under standard operating procedures.

Dr. Wichlacz said this approach has a certain appeal from a regulatory viewpoint since the number value can be quantified and objective. This approach might be combined with use categories.

Dr. Hagedorn said releases from certified facilities differ from field tests. Release from certified facilities may occur through dispersion through a number of different avenues; by aerosol from a vent, on employees' clothing, etc. These emissions, which occur randomly over a period of time, differ from application in a field plot where all of the released organisms will be placed simultaneously into a small area.

Use-Based Approach

Dr. Wichlacz explained the language he and Dr. Summers had developed for a use-based approach to the definition. They originally attempted to list specific uses but quickly realized such an approach would be very difficult if not impossible. In addition, such an approach excluded future uses not now known. They opted instead to base this definition on the development continuum which flows in industry from laboratory scale to pilot scale to prototype to product. Since the NIH Guidelines cover laboratory scale, the next step in the continuum, pilot scale, should trigger EPA review.

Pilot scale is defined as a very small scale test with limited discharge in a single geographic area. The size of pilot scale could differ from industry to industry. Pilot scale in the ore extraction industry is usually about a thousand tons per day. Dr. Finnerty said in the oil refining business pilot scale is 25,000 to 50,000 barrels a day.

Dr. Rissler suggested descriptions of pilot scale for different industries could be listed in a definition. She asked whether greenhouse testing would be considered pilot scale. Dr. Wichlacz replied that would depend on the definition of greenhouse.

Dr. Wichlacz thought under this approach a list of exemptions could be developed and refined as knowledge and experience are gained.

Dr. Summers suggested pilot scale could differ from organism to organism. Dr. Tiedje pointed out that such an approach would bring the element of risk evaluation into the definition.

Control Methods Approach

Dr. Lincoln explained the scheme he and Dr. Milewski developed as an approach to a control-based definition. Their approach involved the use of a point scheme. Points would be allotted to certain types of control measures. Through addition, multiplication, subtraction, etc., points would be summed. At a certain total number of points, the procedure would be considered a release. The proposed language reads:

"The submitter should evaluate the following criteria and assign appropriate number values to the proposed procedure. If the value arrived at through this evaluation is greater than x, the procedure is considered a release reviewable by EPA."

Dr. Lincoln listed some examples of control technologies. Physical control includes barriers (walls, filters, etc.), and chemical control. Biological control might include features such as hardiness, special environmental requirements (such as extreme pH or temperature), and suicide genes.

Organisms which require extremes of pH or temperature to survive would not be likely to affect most environments and so possession of such a characteristic might be viewed as a means of control worth some points in this scheme.

The number of points for each criterion could vary depending on the reliability of the control. For example, if one were confident a suicide gene would effectively control a population, the number of points could differ from the situation where one had limited confidence.

Dr. Lincoln said the list of criteria would have to be further developed by EPA since those listed are examples for the purpose of clarification. EPA would determine which criteria should be included in the list and would have to assign values to the criteria. EPA would also have to determine the trigger point total.

Dr. Lincoln said the scheme allows industry some flexibility in that companies would determine which approach to the point total would make the most sense for the company.

Ms. Hollander said the point scheme should be objective with specific values assigned to the criteria.

Containment Approach

Dr. Hagedorn explained how he and Dr. Albright had tackled their assignment of defining containment. He said they did not have any good description of "contained." They therefore offered some possibilities for discussion. These possibilities included:

"For organisms defined as pathogens, EPA notification and review will precede growth chamber, growth room and/or greenhouse tests, and such growth facilities shall include a level of containment from which no greater than 10^5 organisms per day are emitted.

"For intergeneric and nonpathogenic organisms, EPA notification and review will be prior to conducting any level of evaluation in an unrestricted environment regardless of the size.

"This includes tests on biologically contained organisms until the efficacy of the genetic containment has been demonstrated to be acceptable."

Dr. Hagedorn said the term "unrestricted environment" was used rather than the term "field" because there may be uses where the environment would be unrestricted but would not be a field type application.

Dr. Hagedorn said certain categories of organisms would trigger EPA review: (1) pathogens; and (2) nonpathogenic intergeneric constructs, i.e., organisms constructed from nonpathogenic source organisms from different taxonomic genera. This approach attempts to treat organisms differently on the basis of important properties, even though plant pathologists have been studying plant pathogens in the field for over 80 years and no large epidemics have occurred.

No distinction would be made for organisms which are biologically contained until it has been shown that the biological containment is effective. It may be biological containment is not 100% effective; the data which currently exist are preliminary and suggest it is not.

Dr. Hagedorn suggested EPA may wish to develop a category of "exempt from EPA review" under this approach. The exempt category would apply to certain small scale field trials. Organisms and procedures would be added to the list after data justifying this classification are submitted and reviewed.

Ms. Hollander said the important question in this approach is how the term "unrestricted environment" would be defined generically.

Risk-Based Approach

Dr. Dowler said he and Dr. Finnerty and Mr. Krabbe had been assigned the approach based on risk. They see three groups of factors important in considering risk. These are: (1) biological factors; (2) applications/use factors; and (3) environmental factors. They suggested biological factors have the greatest weight in risk consideration. Examples of biological factors are: the number of organisms released; genetic stability; pathogenicity; survivability; controllability; host range; and origin (indigenous versus exotic). Under this approach, a low, medium and high risk rating would be assigned for each biological factor.

Dr. Dowler offered some general observations on risk ratings. He said a stable organism in most cases would be lower risk than an unstable organism but that might not always be the case. Nonpathogenic organisms probably present a lower risk than pathogens. Organisms which survive a short period of time are less risky than organisms which live for longer periods of time. An organism for which a good method of control exists, whether chemical or biological such as a suicide gene, represents a lower risk. An organism with a limited host range presents lower risk than an organism with a broad host range. If an organism is indigenous to the region in which it is being used, it is lower risk, whereas a new or exotic organism would be higher risk.

Dr. Dowler said application/use factors also play a role in a risk approach. Examples of application/use factors include: manufacturing; biorational (biocontrol); oil recovery; agriculture (pesticide, fertilizer); and metal reclamation. Although these factors would affect the risk rating, the subgroup had not attempted to determine whether the use would be low, medium or high risk.

Dr. Dowler said environmental factors, examples include laboratory, greenhouse, field, commercial production facility, fresh water systems, and marine water systems, refer primarily to where the organism is used. Most laboratory studies are low risk. Greenhouse studies could be a higher risk depending on greenhouse construction. Field application presents a still higher risk. The subgroup did not assign a low, medium or high risk evaluation to these factors.

Dr. Dowler said this approach does not offer a simple definition readily usable by EPA. He thought, however, that some of the other suggested approaches were too simple. He suggested a combination of approaches may be in order.

Dr. Hagedorn said he did not see how application/use or environmental factors could be rated. Several members agreed.

Dr. Dowler thought the point scheme developed by Drs. Lincoln and Milewski could be applied to the risk-based approach. The factors used to compute the point value would be different, however. He liked the idea of giving a numerical value to a factor based on the degree of confidence.

Dr. Dowler suggested a rating scale in a point scheme could have two triggers; one trigger would indicate EPA *could* review the procedure; the second trigger would indicate that EPA *must* review the procedure.

Dr. Rissler thought this risk-based approach would require standardized tests to provide a common basis for comparison.

Organism-Based Approach

Dr. Pierce said the approach based on organisms, which he and Dr. Rouse were assigned, does not offer much latitude to develop a credible definition since this ap-

proach depends on black and white delineations and much in biology is in the grey area. Nevertheless, he and Dr. Rouse were able to list some concepts.

Under this approach, review would be required for certain organisms; for example, *all* pathogens, organisms being placed in a new niche, or for new use of an organism.

For some organisms, review would not be necessary. Examples of organisms suggested for this exempt category include: indigenous saprophytes reintroduced in their niche, organisms incapable of replicating and transferring genes, or when the product is cell or DNA free. Indigenous saprophytes reintroduced into their niche should be shown to be unable to transfer genetic material. Evidence would have to be provided for evaluation prior to inclusion in this exempt category. Even within a category such as pathogens, some organisms will present less of a problem than others and lists of pathogens of different levels of concern could be developed.

Dr. Pierce said the concept of "new niche" is important to this approach and what constitutes a "new" niche would have to be defined.

Dr. Pierce said the difficulty with this approach is that other criteria such as controllability of the organism are also important in defining release but these factors are not considered with an organism-based approach.

Dr. Rissler asked how EPA review would be triggered under this approach. Dr. Pierce said EPA would be notified when experiments are initiated in the laboratory if the ultimate intent is release to the environment.

Dr. Wichlacz said one problem with the approach is that it is driven by an artificial system of classification, taxonomy. This problem may be rectifiable but it should be recognized that the system is not based on evolutionary relatedness. This may present problems when identifying an isolate from the environment.

Dr. Pierce recognized this problem but pointed out that taxonomy is the current standard for identifying and classifying organisms.

Dr. Rouse said a great deal of information exists for many organisms and this can be used to develop this approach.

Identifying Concepts Developed During the Discussion

Dr. Tiedje said he wished to summarize this portion of the meeting by reiterating the important concepts developed in the discussion. The concept of an exempt category was mentioned in three of the six approaches. Field trials of organisms in certain uses would be placed in an exempt category following review of data.

Dr. Tiedje said in the approach based on numbers of organisms released, the subcommittee foresaw difficulty in selecting a number which would credibly encompass all applications.

Dr. Rebecca Goldberg of the Environmental Defense Fund said the approach designating the review trigger as a number of organisms should be considered from the perspective of litigation and public perception: the public might consider the trigger number to be a very large number of organisms.

Dr. Tiedje observed that in the "use-based" approach, the concept of "pilot scale" would have to be better defined and sounds "large-scale" in certain uses.

Dr. Tiedje said the third approach, the control option, requires further development but from a scientific point of view has certain inherent attractions; many of the criteria could be quantified and the scheme offers some flexibility of implementation. A point scheme could also be applied to the risk based approach.

Dr. Tiedje said a definition which relies on a risk assessment raises questions such as: Who would evaluate the "risk factors" in order to know a procedure is a release? Would the company perform such a review itself? Would EPA be involved? The risk-based option did not indicate who would determine whether a procedure was a release.

Dr. Summers said the NIH had addressed that issue by forming Institutional Biosafety Committees (IBCs). He thought IBCs could be used by the EPA to implement a risk based approach to defining release.

Dr. Rissler said some of the approaches appear to suggest levels of containment might be appropriately incorporated in a definition of release to the environment.

At this point in the discussion, the subcommittee recessed until the following morning.

Important Concepts for Evaluating Approaches

Criteria for Evaluating Advantages and Disadvantages of Options

The subcommittee reconvened at 9:00 a.m., December 12, 1986. Dr. Lincoln suggested the group might list some general criteria for evaluating the advantages and disadvantages of each approach. He suggested the following criteria be listed: conditions which might lead to false positives and false negatives; economic burden to Agency and submitter; ease of understanding, interpreting and implementing; information encouraging; and flexibility to incorporate new information, new applications, new industries.

Dr. Tiedje suggested the following criteria might be listed: clearly definable triggers; justification for the trigger; explanation of the trigger; consistency with current information; and credibility.

Mr. Betz suggested the subcommittee might also consider how the approaches are suited to the two EPA statutes; i.e., does one approach make more sense for FIFRA and less for TSCA or vice-versa, or can the approach accommodate both statutes.

Thoughts on Credibility of Approach

Dr. Dowler said in evaluating a definition of release for microorganisms, the more complex the considerations, the more credible the definition from the scientific point of view. However, the more complex the definition, the more difficult to implement and enforce. A "good" definition of release, therefore, must have some degree of complexity to be credible but not so much complexity as to be unenforceable.

Dr. Pierce offered an example of an extreme definition; one that would indicate any release of microorganisms is regulable. From a regulatory standpoint, all procedures and uses would be covered, including those not current-

ly envisaged. However, it is extremely difficult if not impossible to enforce this definition. It has a high cost to both the Agency and industry in terms of time and money, and would generate many false negatives; many procedures which need not be reviewed would be submitted for review. Dr. Rouse said this trigger is not well defined; it could include opening a cork on a test tube filled with microorganisms.

Dr. Finnerty said a definition based on risk is the most complex definition and therefore much more credible than the example offered by Dr. Pierce.

Dr. Lincoln said the control based approach developed on December 11, 1986, represents a middle ground between the approach described by Dr. Pierce and the risk-based options in terms of complexity and ease of implementation.

Further Evaluation of Risk-Based and Approach and Development of a Containment/Organism-Based Approach

Further Evaluation of Risk-Based Approach

Dr. Rouse suggested the subcommittee might break into groups to: (1) further evaluate the control and risk-based approaches; and (2) develop an approach based on containment and categories of organisms.

The subcommittee then divided into two groups: one group which consisted of Drs. Dowler, Finnerty, Lincoln, Pierce and Mr. Krabbe was assigned the task of further evaluating the risk-based and the control-based approaches. The other group which consisted of Drs. Albright, Hagedorn, Wichlacz, Summers and Rouse was to attempt to develop an approach based on organisms and containment.

Dr. Dowler said his group had attempted to rate some of the criteria associated with a risk-based approach. He said the effort was not as easy as the group had anticipated. The criteria were classified as follows: items in category C correlate with risk, are relatively easy to measure and are objective; items in category I are intermediate in correlation with risk, ease of measurement and objectiveness; and items in category P rate poorly in correlating with risk, ease of measurement and objectiveness.

Of the biological factors, pathogenicity and controllability fell into category C; genetic stability, survivability, ability to monitor, and competitiveness with non-target organisms fell into category I; host range and bioproducts released fell into category P.

All of the application/use factors rated by the group fell into category C. These include: aerosol, liquid, above ground, below ground, enclosed/open, growing, non-growing, numbers/volume (concentration).

The three environmental factors cited by the group, site suitability, species, hydrology, fell into category 1.

Approach Based on Containment and Organisms

Dr. Rouse said the approaches based on containment and organisms could be combined into another approach. The types of containment utilized in this approach would include: laboratory, growth chamber, growth room, contained greenhouses, greenhouses, and pilot plants.

Dr. Rouse said the group had envisaged five levels of containment. The highest level of containment would provide the ability to capture most of the organisms. The next level of containment would be similar to the highest level of containment except that the performance standards would be lower. The third category would be a "shielded" type of containment; i.e., a setting buffered from the natural environment by means of some type of physical separation from niches where the organism might be able to grow and survive. The traditional greenhouse might fall into this category. The fourth category would be a setting, such as a field plot, where the released organisms can be mitigated by chemical or physical means. The fifth category would be a field plot restricted by an acreage limitation.

Several general categories of organisms would be developed. Examples of the types of categories of organisms are: human pathogens, animal pathogens, plant pathogens, and saprophytes. Within these categories, subcategories could be created. A saprophytic organism modified by a deletion is an example of a subcategory.

Dr. Rouse said the group had not spent a great deal of time on the classification of organisms; the only distinction they had arrived at was the distinction between pathogens and nonpathogens. They also felt an exemption category for certain organisms in small scale field trials could be developed.

Using containment levels and organism categories, a chart would be constructed. Containment levels would be found on the horizontal axis, while categories of organism would be on the vertical axis. For example, if a nonindigenous plant pathogen which is an obligate biotroph were being developed for a commercial application, EPA review could be triggered at the field test level. On the other hand, if the organism were a nonindigenous obligate biotrophic plant pathogen for which a host is known in this country, EPA review would be triggered at the greenhouse testing level. The point at which EPA review is triggered would be determined by the position of organisms and containment on the chart.

The advantage of this approach is that the trigger is clearly defined. Since the categories of organisms can be moved with respect to containment, it is flexible.

Dr. Rouse said the major problems with such an approach are: can the levels of containment be defined; what is the definition of a greenhouse; the definition of growth chamber; of pilot scale; of field plot. The other major problem is defining the categories of organisms. He felt levels of containment can be defined; he expressed concern that it might be more difficult to define categories or organisms which do not have so many exceptions that the category is useless

Dr. Dowler asked whether this scheme would take into account whether an organism is indigenous. Dr. Rouse replied that it could.

Dr. Tiedje asked whether organisms might also be categorized by nutritional requirement. Dr. Rouse said that suggestion would require a great deal of consideration. He was not convinced such a categorization would make sense.

Dr. Rissler asked how applications which are not tested in field plots would be handled under the scheme. Would EPA have to devise an industry by industry chart? Dr. Rouse suggested analogies between "field plot" and "pilot scale" could be made.

Dr. Segal said the organism/containment approach contains one element of hazard evaluation; hazard evaluation is implicit in the attempt to categorize organisms.

Dr. Tiedje said this scheme could become quite complex as the number of categories of organisms and levels of containment increase. He pointed out that the more complex the definition the more time EPA will need to fully develop and implement the approach. He pointed out that the number of criteria in the risk-based approach is quite large and the number of criteria in the organism/containment approach could also be high. He thought at some point a large number of criteria would render an approach inoperable. He said these two approaches are data intensive for both the Agency and industry.

Dr. Lincoln said these two approaches are data intensive both in developing criteria and in getting the criteria to mesh. There is some difference, however, between the two approaches. The organism/containment approach is intensive initially, i.e., there is an up-front effort to prepare the chart. There may be some continuing effort needed to accommodate different uses and organisms. The risk-based approach appears to require a continuing effort from both industry and the Agency.

Adjournment Dr. Tiedje thanked the subcommittee and observers for participating in this effort. He adjourned the meeting at 4:00 p.m. on December 12, 1986.

Respectfully submitted,
Elizabeth A. Milewski Ph. D.
Executive Secretary

I hereby certify that, to the best of my knowledge, the foregoing minutes and attachments are accurate and complete.

May 23, 1987

James Tiedje, Ph. D.
Chair
Subcommittee on Definition of Release to the Environment of the Biotechnology Science Advisory Committee

Appendix 3

Materials Supplied to the Subcommittee

- The June 26, 1986, *Federal Register* (51FR23302) which contains EPA's policy statement for addressing, under TSCA and FIFRA, certain microbial products of biotechnology.

- "Assessing Physical Containment in Recombinant DNA Facilities" by E. Fisher and D. Lincoln. This paper describes a computer simulation model that has been developed to assess by simulating microorganism movement the effectiveness of various physical containment strategies. Simulations were used to assess several aspects of containment, including the effects of using different protocols and host organisms, establishing tradeoffs between physical and biological containment levels, and recognizing the variability introduced by human error and equipment failure.

- "Studies on Release and Survival of Biological Substances Used in Recombinant DNA Laboratory Procedures" by M.A. Chatigny, M.T. Hatch, H. Wolochow, T. Adler, J. Hresko, J. Macher and D. Besemer. This paper provides information on the efficacy of laboratory containment systems. It examines the numbers of organisms produced by aerosols or spillage incidents in the laboratory. It attempts to evaluate the survival of *E. coli* strains in aerosols and on various surfaces.

- "Defining an 'Environmental Release' of Microorganisms: Consequences of Tying the Definition to Emissions from a 'Typical' BL1 Laboratory" by H. Strauss. This paper was prepared for EPA under a Cooperative Agreement between EPA and the Center for Technology, Policy and Industrial Development of the Massachusetts Institute of Technology.

The number of organisms most likely released from a "typical" BL1 laboratory is compared to the numbers of organisms expected to be released from various uses. The uses examined to determine whether or not they would result in an "environmental release" if a BL1 laboratory were the standard for "contained" include: greenhouses; agricultural field trials; mineral leaching; coal or oil desulfurization; microbially enhanced oil recovery; spill/waste cleanup; sewage treatment works; and, microchambers in streams. With the exception of microchambers in streams, these uses appear likely to emit, in some circumstances, greater numbers of microorganisms than those emitted from a BL1 laboratory per day.

- "The Survival of Host-Vector Systems in Domestic Sewage Treatment Plants" by B.P. Sagik and C.A. Sorber. This paper provides information on the survival of certain *E. coli* strains in sewage treatment plants.

- "Ambient Exposure Assessment Methods for Intentional Releases of Recombinant Microorganisms," a draft report prepared by Versar, Inc., for the EPA. This report at the time of the meeting had not undergone a full technical and editorial review and has not yet been formally released by EPA.

The purpose of this report is to present methods for assessing ambient exposures; i.e., exposures in the open environment, of microorganisms intentionally released for commercial applications. The methods are based on a stepwise development of information on the source, release and environmental fate of the organisms.

Four scenarios in which organisms are applied in the environment are examined. These scenarios were selected to cover a wide range of industrial uses and affected habitats, so that the methods could be tested for flexibility and breadth of application. Mathematical models developed to estimate the exposure component of a risk assessment for chemicals are applied to these four scenarios and tested for applicability to biological

scenarios. The exposure calculations utilize quantifiable physical transport characteristics such as source, emission and transport properties. The models used in the report depend on the assumption that microorganisms behave like or are closely associated with, a physical unit for which the model is calibrated such as aerosols and particulates for air modeling, or soil particles for storm runoff. "Die-off" in microbial populations is considered to be analogous to physical transformation processes that result in "decay" of a dispersed chemical with distance over time.

Ecosystem characteristics were also included in the exposure assessments. These include: topography/bedrock profiles, soil, weather and climate, hydrogeology, land use data, surface water parameters, local ground-water chemistry and habitat.

Several ecological processes which can only currently be included in the model in qualitative terms were included in the analysis of the transported microorganism: competition, antagonism, predation and synergism.

Report of the Meeting of the Biotechnology Science Advisory Committee; Subcommittee on Definition of Pathogen

February 26-27, 1987

Introduction

Although biotechnology is thousands of years old, several concerns have been raised over the past 15 years by the use of the techniques (recombinant DNA, cell fusion, etc.) which have been called the "new" biotechnology. Because these "new" techniques permit precise manipulation of the genetic inheritance of organisms and circumvention of species barriers, these techniques are very powerful.

One of the concerns associated with the use of the techniques known as the "new" biotechnology is that "hazardous" microorganisms might be produced through these techniques. Although at this time this concern is conjectural, it has been hypothesized that such an outcome may be more likely in some circumstances than in others, and such circumstances have generally been subjected to greater scrutiny. One circumstance where it is hypothesized such an outcome may be more likely is when the parent organisms (i.e., organisms used as hosts or donors in constructing microorganisms to perform specific tasks) are pathogens, since pathogens have the intrinsic potential to cause adverse effects.

Pathogens are microorganisms that cause disease in other organisms (human, animal, plant or other microorganisms), with "disease" being defined as an abnormal physiological process in an organism. These abnormal physiological processes encompass a broad range of effects such as oncogenicity, teratogenicity, mutagenicity, altered immune responses, and interruptions in cellular metabolism. There are several mechanisms whereby microorganisms can induce such adverse effects

One such mechanism is when the proliferating microorganisms grow in or on the target organism's tissues and directly affect the target organism. For example, some pathogenic organisms produce toxins, other pathogenic microorganisms produce enzymes that degrade tissue.

Microorganisms can also cause deleterious effects when they grow outside of the target organism but produce organic toxins which can work "from a distance." An example of this mechanism is a disease of chrysanthemums called strap leaf. This disease is caused by a toxin produced by *Aspergillus* growing in the soil.

Another way microorganisms can harm other organisms is by altering the habitat and thereby indirectly affecting other populations also occupying that habitat. One example of this phenomenon arises when there are population imbalances within the microbial community. For example, large influxes of nutrients can lead to large algal blooms in lakes. These algal blooms produce anoxic conditions which can lead to disease symptoms and death in oxygen-requiring populations such as fish. In this case, the adverse effects are due to anoxia caused indirectly by microbial populations. Such indirect effects may also be produced by microbial populations which produce inorganic chemicals that can cause abnormal physiological processes in other organisms. For example, hydrogen sulfide produced by microbial populations in sediment can accumulate to concentrations toxic to other organisms.

It has been suggested that another circumstance which might deserve regulatory scrutiny is applications involving microorganisms controlling important biogeochemical cycles, such as nutrient cycling.* Changes in biogeochemical processes are of concern because these important processes greatly influence the homeostasis of the planet and, it has been hypothesized, adverse effects could ensue if the balances between microorganisms playing a major role in these processes are disrupted. An example of an important element cycle is the nitrogen cycle. Nitrogen plays an essential role in living organisms and is a key element limiting growth. Although nitrogen is an abundant atmospheric gas, this form is not directly usable by the majority of organisms. Only certain species of bacteria and blue-green algae possess the ability to reduce and fix atmospheric nitrogen.

The importance of these organisms in maintaining a balance between fixed and atmospheric nitrogen is seen in the following. In oceanic or terrestrial ecosystems, where nitrogen is the major limiting nutrient, gaseous loss of nitrogen, if it is not compensated by organisms capable of fixing nitrogen, would reduce the nitrogen available for living organisms. On the other hand, an increase of 1% in the emission rate of N_2O would cause a 0.2% decrease in stratopheric ozone, increasing the transmissivity of ultraviolet light through the atmosphere.

As part of its effort to address concerns about "potentially hazardous" microorganisms, the Environmental Protection Agency (EPA) has convened a group of experts to discuss which groups of microorganisms should, because these organisms might possess an intrinsic potential to cause adverse effects, be subjected to a greater regulatory scrutiny.

History

A brief review of the history of recombinant DNA, one of the techniques of the "new" biotechnology, is included in this report to show how the concept of "pathogen" has influenced the oversight of recombinant DNA technology. It was the scientists who engaged in research using the technique termed recombinant DNA who first called for assessment of the risks which might be associated with this technique, and for guidelines which would permit research to proceed in a cautious manner. The National Institutes of Health (NIH) was asked to develop these guidelines. In July 1976, NIH published its Guidelines for Research Involving Recombinant DNA Molecules.[1] In this original version of the guidelines, certain experiments were "not to be initiated. . . ." Included in this group

*Nutrient cycling refers to the fluxes of elements and other nutrients in the ecosystem.

were experiments involving the use as hosts, or the cloning of DNA from, organisms classified as Class 3, 4, and 5 pathogens in the "Classification of Etiologic Agents on the Basis of Hazard."[2]

The "Classification of Etiologic Agents on the Basis of Hazard" was developed by the Center for Disease Control (CDC) and lists the following classes of animal and human etiologic agents:

Class 2—agents of ordinary potential intrinsic hazard;

Class 3—agents involving special intrinsic hazard;

Class 4—agents that require the most stringent conditions for their containment because they are extremely hazardous to laboratory personnel or may cause serious epidemic disease; and

Class 5—animal pathogens that are excluded from the United States by law or whose entry is restricted by U.S. Department of Agriculture administrative policy.

Class 1 agents are not listed in the CDC "Classification" but are assumed to be organisms that are not listed in Classes 2 through 5. Class 1 agents are assumed to present no or minimal hazard under ordinary conditions of handling.

The "Classification" primarily deals with agents etiologic in humans. NIH's rationale for using this classification is described in the NIH "Environmental Impact Statement on NIH Guidelines for Research Involving Recombinant DNA Molecules"[3] which notes that:

"the use in the Guidelines of the CDC Classification is based on the recognition that the laboratory worker conducting recombinant DNA research has the highest potential for exposure, and is the most likely route for transmission of microorganisms to other living things."

The first revision of the guidelines which appeared in 1978[4], prohibited experiments involving organisms classified as Class 3, 4 or 5 pathogens. Individual waivers could, however, be approved by the NIH Director following publication of proposals for public comment in the *Federal Register* and review and approval by the local review committee, the Institutional Biosafety Committee (IBC).

An appendix composed of the "Classification of Etiologic Agents on the Basis of Hazard" and the "National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses," was included in the 1978 version of the Guidelines. The National Cancer Institute Safety Standards is a listing of low and moderate risk oncogenic viruses.

In the 1982 version of the Guidelines[5], experiments involving Class 3, 4 or 5 pathogens were no longer prohibited. The Guidelines permitted these experiments provided that IBC review and approval were obtained before initiation of these experiments. The Guidelines specified the containment that should be employed when these organisms were used. Experiments involving Class 2 organisms as the recipient organism were to be performed at P2 (now termed BL2) containment; experiments involving Class 3 organisms as the recipient organism were to be performed at P3 (now termed BL3) containment; experiments involving Class 4 organisms as the recipient organism were to be performed at P4 (now termed BL4) containment. Conditions for experiments involving the introduction of recombinant DNA into Class 5 agents were to be determined on a case-by-case basis.

The BL levels are biosafety levels that consist of combinations of practices and techniques, safety equipment, and laboratory facilities appropriate for the operations performed and the hazards posed by agents and for the laboratory function and activity. BL level 4 provides the most stringent containment while BL level 1 is the least stringent. BL1 is considered to be good microbiological technique.

Although the NIH system of oversight of research worked well for ten years, as the number of commercial applications increased, another mechanism of providing regulation/oversight was implemented by the Federal government. In 1984, the government formed an interagency group under the White House Cabinet Council on Natural Resources and the Environment. That group developed a coordinated regulatory framework[6] for products of biotechnology.

Within this coordinated framework, two EPA statutes have been interpreted as investing the Agency with the authority to regulate biotechnology products. These statutes are:

- The Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) creates a regulatory framework under which EPA regulates the sale and distribution of pesticides. FIFRA gives EPA oversight responsibility over microbial pest control agents whether or not these agents are genetically engineered.

- The Toxic Substances Control Act (TSCA) authorizes EPA to acquire information on "chemical substances" and "mixtures" of chemical substances in order to identify potential hazards and exposures. TSCA gives EPA jurisdiction over the manufacturing, processing, distribution, use and disposal of all chemicals in commerce or intended for entry into commerce that are not specifically covered by other regulatory authorities. TSCA's applicability to regulating microbial biotechnology products is based on the interpretation that microbes are chemical substances under TSCA.

EPA'S Approach

As part of the coordinated framework, EPA issued a *Federal Register* notice on June 26, 1986, explaining how certain products of biotechnology would be regulated. The June 26 Federal Register indicated that EPA under FIFRA and TSCA intends to focus on three categories of microorganisms as deserving a higher level of regulatory scrutiny and emphasis. These are:

- "pathogens" and organisms constructed from pathogens because of the inherent ability of pathogens to cause disease. The definition of pathogen employed in the June 26 *Federal Register* is ". . . a virus or organism (including its viruses and plasmids, if any) that has the ability to cause disease in other living organisms (i.e., humans, animals, plant, or microorganisms). A disease

is an abnormal physiological function in an organism, occurring as a consequence of the activity of proliferating microorganisms directly associated with or infecting the host organisms, or due to biologically active substances such as toxins, antibiotics or growth regulators produced by the organism . . .";

- microorganisms with "new" characteristics or that are "new to the environment" in which they will be used (where "new" is defined as organisms constructed from source organisms from different genera and where "new to the environment" is when the microorganism is isolated from outside certain defined geographical areas); and
- microorganisms that are used in the environment.

The June 26 *Federal Register* made certain policies immediately effective and others, which will be made effective through the rulemaking process, voluntary.

A summary of the current policy under TSCA and FIFRA for review of microorganisms released into the environment is included in the Appendix as Table 1.

Implementation under TSCA. The portion of EPA's policy under TSCA dealing with pathogens is currently voluntary and will be fully implemented through the rulemaking process. To implement this portion of the policy under TSCA, EPA is writing a "significant new use rule" (SNUR). The SNUR is the statutory mechanism that allows EPA to review exposure and toxicity information prior to manufacture, importation, distribution, processing, use or disposal of a substance covered by TSCA. Microorganisms which would be subject to TSCA include those used in conversion of biomass for energy, pollutant degradation, sewage treatment, enhanced oil recovery, metal extraction and recovery, fossil fuel desulfurization processes, and certain non-food and non-pesticidal agricultural applications, such as nitrogen fixation.

When EPA has promulgated its SNUR for organisms covered by the rule, manufacturers or developers must submit a SNUR notice to the Agency ninety days before the planned release to the environment. Upon receipt of a SNUR notice, the Agency will conduct a risk/benefit review and take any appropriate regulatory action. Such review insures that EPA can take action, if necessary, to prevent potentially hazardous exposure of persons or the environment to the substance.

The definition which describes the organisms covered by the SNUR will determine the scope of EPA coverage within the SNUR; significant new uses of microorganisms subject to the SNUR provisions will be subject to the higher level of regulatory scrutiny.

The Agency has also determined that section 8(a) of TSCA would require that some information on microorganisms not covered by the SNUR be reported to the Agency. Therefore, investigators not subject to the SNUR could still be required to report some information under TSCA section 8(a).

Implementation under FIFRA. FIFRA defines a pesticide as "(1) any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest, and (2) any substance intended for use as a plant regulator, defoliant, or desiccant." Most microbial pesticides may be considered pathogens because by design they adversely affect living organisms. While the definition of "pathogen" is not essential to defining the scope of FIFRA coverage, it is used for determining the level* of review prior to small-scale field testing of microbial pesticides. (Small scale is defined as <10 acres of land or <1 acre of water.) Thus, the definition of "pathogen" will determine the type of review a microbial pesticide covered by EPA's policy will receive prior to small-scale field testing.

The Meeting

Format

In order to utilize a broad spectrum of expertise in developing a final policy for microorganisms subject to EPA's regulations, EPA assembled a group of recognized technical experts as a subcommittee of the Agency's Biotechnology Science Advisory Committee (BSAC). This subcommittee, the Subcommittee on Definition of Pathogen, met in public session on February 26-27, 1987, in Arlington, VA. Attached to this report are minutes of the meeting (Appendix 1). A subcommittee roster is included in the minutes as Appendix 2.

On the first day of the meeting, the issues and EPA's goals were explained. Specific comments and observations were solicited from each participant from the perspective of their expertise and research experience on the issues before the subcommittee. The group was then asked to "brainstorm" and attempt to suggest as many approaches as possible. The subcommittee was asked at this point not to attempt to judge the acceptability or credibility of the approaches. The subcommittee would comment on the advantages and disadvantages of the approaches later in the meeting.

After several approaches were suggested, subcommittee members were assigned to smaller groups to develop the proposed approaches.

The subcommittee was told that from the Agency's perspective the most important characteristics of a "good" definition were:

- Simplicity and ease of interpretation. It is advantageous to both the Agency and industry to be able to determine easily and readily whether a particular product is covered by EPA regulations. Ideally, both EPA and the producer should be able to make the determination on the basis of existing data. In other words, it would be undesirable to have a definition that would require testing in order to determine whether the product would fall within the regulated category.
- Credibility. The definition should be based on scientific principles.
- Flexibility. The definition should be applicable to situations which may develop in the future but are not currently envisaged.

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*There are two levels of review of microbial pesticides covered by EPA's policy prior to small-scale field testing. Level II review requires more detailed information and in-depth review than level I.

Examples of possible approaches were also offered to the subcommittee. Included were the following examples:

- a list of traits;
- a definition based on specific host groups (e.g., pathogens of plants, animals and humans);
- a listing of biologically significant properties of organisms or groups of organisms; and
- exclusion of specific groups of organisms (e.g., opportunistic pathogens).

It was emphasized that the subcommittee was not limited to these suggestions, but was encouraged to develop a variety of approaches.

Objectives
The Agency asked the subcommittee to provide several approaches to defining the microorganisms that should receive regulatory scrutiny.

EPA also asked the subcommittee to explain the advantages, disadvantages, limitations and implications of each approach.

Approaches
Five approaches to describing the organisms that would be subject to the higher level of review were suggested by the subcommittee. The approaches were based on: (1) modifications to the current EPA definition published in the June 26, 1986 *Federal Register;* (2) categories of hosts and microorganisms; (3) lists of microorganisms; (4) traits of microorganisms; and (5) a "process-based" approach.

1. *Modifications to Current EPA Definition*
Several variations on the definition of pathogen that appeared in the June 26 *Federal Register* were proposed. In order to permit the reader to easily compare these variations with the current EPA definition, the definition and the pertinent sections of the policy published in the *Federal Register* are included in this report. The June 26, 1986 definition is as follows:

"For the purposes of this policy, a pathogen is defined as a virus or organism (including its viruses and plasmids, if any) that has the ability to cause disease in other living organisms (i.e., humans, animals, plants, or microorganisms).

A disease is an abnormal physiological function in an organism, occurring as a consequence of the activity of proliferating microorganisms directly associated with or infecting the host organisms, or due to biologically active substances such as toxins, antibiotics or growth regulators produced by microorganisms."

The policy statement also explained that:

"This policy is not meant to include such organisms as competitors or colonizers of the same substrates, commensalistic or mutualistic microorganisms, or opportunistic pathogens. However, if a microorganism has more than one mechanism for affecting other organisms and one of these is pathogenicity, then the microorganism is considered to be a pathogen.

"A microorganism will be subject to EPA policies if:

- The organism belongs to a pathogenic species or to a species containing pathogenic strains, according to sources identified by EPA . . .
- The organism has been derived from a pathogen or has been deliberately engineered such that it contains genetic material from a pathogenic organism. . . . An exception to this requirement is a genetically engineered organism developed by transferring well-characterized, non-coding regulatory regions from a pathogenic donor to a nonpathogenic recipient. . . ."

The policy statement further explained that the geographic point of isolation would affect the status of organisms. The policy states that:

". . . given the complexity and impracticality of determining whether a particular microorganism is indigenous to a wide range of habitats that may exist within regions and states, the Agency has selected continental boundaries to describe geographic regions that are clearly isolated and are easily used for administrative purposes. These boundaries will be used to determine whether a microorganism is nonindigenous and hence subject. . . ."

Variations. The variations to this definition and the policy statement suggested by the subcommittee are:

Variation #1—The distinction between indigenous and nonindigenous origins of microorganisms would be eliminated from the policy.

Variation #2—The scope of the policy would be expanded to include opportunistic pathogens in the category of organisms receiving greater regulatory scrutiny. It is difficult to define the distinction between opportunistic and frank pathogens. This is particularly true for human and animal pathogens.

Variation #3—Under this variation the scope of the policy would be modified to include opportunistic pathogens of animals and humans. Opportunistic pathogens of plants would not be included among the organisms receiving greater regulatory scrutiny.

Variation #4—The phrases "including viruses and plasmids" and "microorganisms as hosts" would be eliminated from the definition.

Variation #5—The phrase "pathogens of microorganisms" would be deleted from the definition and language describing undesirable effects microorganisms might have on other microorganisms would be included in the policy statement.

Variation #6—"A pathogen is a virus or a microorganism, including its viruses, bacteriophages, transposable elements, and plasmids, which can cause debilitating, disfiguring, destructive, or lethal effects upon the anatomy, tissues, organs, physiology, development or behavior of a host organism. . . ."

Variation #7—(Changes to current definition italicized). A pathogen is a virus, *viroid,* or organism (including its viruses and plasmids, if any) that has the ability

to cause *an adverse effect on the physiological processes of* other living organisms (i.e., humans, animals, plants or microorganisms). *Pathogens may parasitize the host organism directly and cause an adverse disruption or cause an adverse disruption through the production of biologically active substances* such as toxins, antibiotics, or growth regulators.

This policy is not meant to include organisms involved in commensalistic or mutualistic associations, or opportunistic pathogens. *An opportunistic pathogen (for this policy) is one that can only parasitize severely weakened hosts and will not cause an adverse effect in healthy individuals.* Pathogens include:

- Pathogenic species or species with pathogenic strains
- Microorganism products of biotechnology with genetic material from pathogens.

Exemptions include:

- *Known nonpathogenic strains used for laboratory research or commercial purposes only, e.g., E. coli K-12*
- *Microorganism products of biotechnology constructed by introducing genetic material from pathogen into nonpathogen recipient where genetic material is well characterized and*

—a coding region not involved in pathogenicity of donor or recipient

—a noncoding regulatory region from a pathogenic donor to a nonpathogenic recipient.

2. *Categories of Hosts and Microorganisms*
This approach would consist of "categories" of microorganisms and hosts. The status of the various categories under the EPA policy would be described by the Agency.

In the example developed by the workgroup, the following categories of microorganisms would be subject to a higher level of scrutiny.

"• Frank Pathogens

—Indigenous frank pathogens of humans, animals and non-insect invertebrates would be subject to the higher level of review at the small scale field testing level.*

—Indigenous frank pathogens of insects and plants would *not* be subject to the higher level of review at the small scale field testing level. They would be subject at the large scale testing level.*

—Non-indigenous frank pathogens would be subject to the higher level of review at the small scale field testing level.*

"• Toxin producers, mutagens, carcinogens

"• Microorganisms that have received, by human intervention, a gene(s) for toxin production

"• Microorganisms converted to pathogens by addition of genes by human intervention

"• Microorganisms that have received uncharacterized genetic material from pathogens (and non-pathogens?)

"• Microorganisms which mediate specific changes in rates or pathways of degradation, mobilization or synthesis, within ecosystems. Such microorganisms may be grown in quantity without genetic alteration or they may be created through artificial selection, mutagenesis or techniques of recombinant technology

"• Microorganisms that are specifically developed or modified to degrade chlorinated hydrocarbons (e.g., dioxins). The Agency would be reviewing for additional effects (e.g., high rates of lignin degradation)."

The workgroup also developed an example of categories of organisms which might not be subject to the higher level of review scrutiny under TSCA:

"• Nonpathogens

"• Saprophytes (except those described as receiving the higher level of review)

"• Organisms that are allergenic

"• Opportunistic pathogens

"• Frank pathogens of microorganisms (protists, fungi, bacteria)

"• Microorganisms receiving genes from pathogens by human intervention, which are not involved in a toxin production or do not convert microorganisms to a pathogen."

Comments on this Approach. Mechanisms by which this approach would be implemented would have to be developed. A mechanism of implementation for this approach could be a list. In order to implement this approach, several terms have to be defined; e.g., frank pathogen, opportunistic pathogen and toxin producer.

3. *Lists of Microorganisms*
This approach would consist of lists of microorganisms. For example, one approach would consist of a list of pathogens which are subject to the higher level of review and a list of nonpathogens which would not be subject to the higher level of review. If a microorganism is not on either list, the Agency would be contacted to determine the status of the microorganism.

The lists should: (1) be illustrative guides rather than being comprehensive or all encompassing; (2) be dynamic; and (3) list microorganisms by strain.

Comments on this Approach. Academia, industry and scientific societies, representing the various microorganism classifications (bacteria, fungi, etc.) as well as host classifications (mammals, invertebrates, fish, plants, etc.) could assist in compiling the lists. EPA would make the final decision as to how or whether organisms would be listed.

Several advantages are associated with this approach (1) The approach meets the need of industry and the Agency for guidance. (2) Since the lists would be dynamic, the approach anticipates changes and evolution of knowledge. (3) If several groups (EPA, scientific societies,

*Field tests conducted on <10 acres of land or <1 acre of water are considered smallscale.

academia, industry) participate in the effort to generate the lists, communication links between various constituencies would be built. Furthermore, updating of the lists would be easier since the initial mechanism for generating the lists would be in place. (4) The mechanism of implementation is straightforward. (5) This approach could be used in conjunction with other approaches.

This approach also has several disadvantages. (1) Generating and maintaining the lists would be time-consuming and labor intensive. (2) The list might be "taken as gospel," i.e., be perceived as a "definitive statement" rather than as a mechanism to implement EPA's policy. Rather than being dynamic, illustrative guides, the lists might become difficult to update and change. (3) The approach might tend to channel research, since submitters would tend to choose to use microorganisms on the basis of the listing. (4) Finally, there are inherent difficulties in creating a dynamic list: Incorporation of advances in knowledge could create confusion due to the changing status of the microorganisms on the list. Changing the list could affect the status of organisms being developed for commercial applications (i.e., microorganisms which were subject on an earlier list might not be subject on an updated list).

Questions the Agency would have to address, if it chose to use this approach, are: What would a submitter do if the status of the submitter's microorganism changed during the course of research and development and how frequently would the submitter need to check its status? Furthermore, how will organisms which are not subject to review when put into commercial production but whose status later changed to "subject" be handled?

Another issue is how many lists would have to be created to capture all of the microorganisms which should be reviewed. Should there be just one list, a list of frank pathogens; or should other lists be developed to address several different categories of "microorganisms of concern"; or should lists of pathogens be used in conjunction with lists of nonpathogens to determine the status of organisms?

It may prove difficult to compile a list of nonpathogens, given the current level of knowledge. Microorganisms which are known to be nonpathogens for humans and plants might be pathogenic to less well-studied organisms such as invertebrates.

Another issue is whether "opportunistic" pathogens should be included on a list. Opportunistic pathogens present the issue of defining how they differ from frank pathogens. The delineation between frank and opportunistic pathogens is not clear for human and animal pathogens although the distinction is more easily described for plant pathogens.

The issue of how to deal with organisms constructed by introducing genetic material from a pathogen into a nonpathogen is not addressed in this approach.

One suggestion that might reduce some of these concerns is that the list(s) could concentrate on microorganisms most likely to be used in the near future. The list(s) could be further divided into categories based on host organisms.

4. *Traits of Microorganisms*

The workgroup suggested language which describes "traits" of microorganisms to be used to characterize organisms subject to the higher level of review. As an example they suggested the following language:

". . . microorganisms possessing the following traits are covered:

"• Microorganisms possessing mechanisms of infectivity, e.g., the ability to recognize a target surface, the ability to attach to a target surface, and the ability to penetrate a target surface.

"• Microorganisms having a basic compatibility with the host; i.e., the microorganism uses host nutrients and either activates or bypasses host defense mechanisms.

"• Microorganisms possessing characteristics which affect the host; e.g., toxins, hydrolases, proteases, growth regulators, inducing a deleterious effect, response or death. This includes factors that act at a distance, such as toxins.

"• Microorganisms having mechanisms of dispersal to other hosts.

"• Microorganisms having an ability to survive by resisting environmental stress or producing large enough numbers of progeny to successfully maintain its population."

Possession of some combination of these five traits would cause a microorganism to be subject to the higher level of review scrutiny. Some traits could have an "absolute weight" such that possession of that trait would cause a microorganism to be subject; e.g., possession of an ability to induce "a deleterious effect, response or death." Conversely, possession of only a single trait could exempt the microorganism, e.g., lack of the ability to disperse.

Comments on this Approach. The advantages of this approach are: (1) The word "pathogen" would not be used; rather, possession or absence of certain traits would trigger review. (2) Avirulent forms would not be subject. (3) No distinction would be made between genetically engineered and naturally occurring microorganisms. Any combination of genetic material (independent of the source) which leads to the above traits or subset of traits would be subject. (4) Traits of concern could be rather explicitly identified. (5) Nonpathogens which had received well-characterized sequences from a pathogen might not be covered if the introduced sequences do not code for the traits. (6) The traits are testable.

The disadvantages of this approach are: (1) Submitters would need to know whether the microorganisms possessed the listed traits in order to determine subjectability; the submitter may therefore have to conduct some testing. (2) This approach does not address "nuisance" organisms such as algae growing in waterways. (3) Unless the language is carefully crafted, many false positives and negatives might be generated.

Two additional traits which might be part of this approach were also suggested: the "capacity to change

(modify, expand, shift) host range or virulence by relatively simple mutations"; and "the potential for restoration of function if avirulence is due to a single gene mutation."

TSCA section 8(a) reporting requirements will state that manufacturers, importers and processors would be required to report some information on microorganisms not subject to the higher level of review. This would constitute a "safety net" to capture organisms which may be incorrectly identified as not subject to the higher level of review.

5. *"Process-Based"*

During the subcommittee meeting, it was also suggested that a "process-based" approach to implementing a trigger for the higher level of review would be feasible: "any microorganism which has been modified using recombinant DNA techniques would be subject to the higher level of review scrutiny."

Comments on this Approach. The advantage of this approach is that it addresses the public's primary concern in a very direct manner, and is the most simple and direct response to the issues faced by the Agency.

The disadvantage of this approach is that the method of construction rather than the effects caused by the microorganism would be the primary consideration in determining whether the microorganism should be regulated. The potential effects of microorganisms are, however, the primary reasons for establishing oversight, and at what level, over microorganisms. There is no reason to assume that genetically engineered organisms by definition pose greater risks than other microorganisms.

References

1. Guidelines for Research Involving Recombinant DNA Molecules, *Federal Register,* 1976.

2. Classification of Etiological Agents on the Basis of Hazard, 4th edition, July, 1974, U.S. Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, Office of Biosafety, Atlanta, Georgia.

3. National Institutes of Health Environmental Impact Statement on NIH Guidelines for Research Involving Recombinant DNA Molecules, October, 1977.

4. Guidelines for Research Involving Recombinant DNA Molecules, *Federal Register,* 43, 60108, 1978.

5. Guidelines for Research Involving Recombinant DNA Molecules, *Federal Register,* 47, 38048, 1982.

6. Coordinated Framework for Regulation of Biotechnology; Announcement of Policy and Notice for Public Comment. *Federal Register,* 51, 23313, 1986.

Appendix 1

Minutes of the Meeting*

The Subcommittee on Definition of Pathogen of the Biotechnology Science Advisory Committee (BSAC) was convened at 9:00 a.m. on February 26, 1987, in room 1112 in Crystal Mall 2, 1921 Jefferson Davis Highway, Arlington, VA 22202. The meeting recessed at 5:00 p.m. on February 26, 1987, and reconvened on February 27, 1987, at 9:00 a.m. The meeting was open to the public. Dr. Robert McKinney was Chair. A subcommittee roster follows. The following people were present for all or part of the meeting.

Subcommittee Members:
Elizabeth Davidson
John Harschbarger
Conrad Istock
Michael McGinnis
Robert McKinney
Margaret Mellon
Svend Nielsen
Douglas Rouse
Luis Sequeira
Frieda Taub
Elizabeth Milewski (Executive Secretary)

EPA Support Staff:
Janet Andersen
Ann Huang
Jane Rissler

Others:
Jodie Bakst, Environmental Protection Agency
Susanne Baskin, Environmental Protection Agency
Fred Betz, Environmental Protection Agency
Charlene Dunn, Environmental Protection Agency
Charles Eby, Monsanto
Jeffry L. Fox, ASM News and Bio/Technology
Bob Frederick, Environmental Protection Agency
Anita Glazer, Arthur D. Little, Inc.
Alan Goldhammer, Industrial Biotechnology Association
Ron Grandon, PTCN
Mary Gant, Office of Science and Technology Policy
Georgia Helmick, Ciba-Geigy Biotech
Carol L. Hoerner, Genentech, Inc.
Cheryl Hogue, BNA Chemical Regulation Reporter
Richard Hufer, University of Maryland
Stan Janusz, Dynamac Corporation
Daniel Jones, U.S. Department of Agriculture
Y'Vonne R. Jones-Brown, Dynamac Corporation
Jan Kurtz, Environmental Protection Agency
Morris Levin, Environmental Protection Agency
James H. Maryanski, Food and Drug Administration
Jack McCarthy, Environmental Protection Agency
Henry Miller, Food and Drug Administration
Richard Parry, U.S. Department of Agriculture
Thomas Parshley, Ciba-Geigy Corporation
Kim Pham, Environmental Protection Agency
Christine M. Riley, Beveridge and Diamond, PC
Louis W. Roberts, Department of Transportation
Marvin Rogul, University of Maryland
J. David Sakura, Arthur D. Little, Inc.
Philip Sayre, Food and Drug Administration
William R. Schneider, Environmental Protection Agency
Mark Segal, Environmental Protection Agency
Janet Shoemaker, American Society for Microbiology

*The Subcommittee is advisory to the BSAC which is advisory to the EPA. Its recommendations should not be considered final or accepted.

Roy Sjoblad, Environmental Protection Agency
Art Stern, Environmental Protection Agency
William Szkrybalo, Pharmaceutical Manufacturers Association
Greg Theis, Environmental Protection Agency

Call to Order and Introductory Remarks

At 9:00 a.m. on February 26, 1987, Dr. Robert McKinney, Chair, called the meeting to order. He welcomed the participants.

Dr. McKinney said he intended to provide time to the public to comment but reserved the right to interrupt commenters who do not address the topic or who utilize more time than is prudent given the tight agenda.

Dr. McKinney asked the members of the subcommittee to introduce themselves. Dr. McKinney is Chief of the Occupational Safety and Health Branch at the National Institutes of Health (NIH). He was a member of the NIH Recombinant DNA Advisory Committee (RAC) from 1981 to 1985, and participated in developing Appendix K of the NIH Guidelines for Research Involving Recombinant DNA Molecules. Appendix K details large scale containment specifications. He also participated in preparing the booklet "Biosafety in Microbiological and Biomedical Laboratories."*

Dr. John Harschbarger of the Smithsonian Institution said he is Director of the Registry of Tumors in Lower Animals. His primary interest is cancer in cold-blooded vertebrates and invertebrates. He is President of the Society for Invertebrate Pathology.

Dr. Douglas Rouse of the University of Wisconsin said he is a plant pathologist with a special interest in epidemiology. He has extensively studied two disease systems; diseases of potatoes caused by soil borne pathogens and bacterial diseases of beans.

Dr. Svend Nielsen of the University of Connecticut said he is a veterinary pathologist. He diagnoses and studies diseases of domestic and wild mammals.

Dr. Michael McGinnis of North Carolina Memorial Hospital said he is a mycologist and bacteriologist. His special interests are opportunistic pathogens and biosystematics.

Dr. Freida Taub of the University of Washington said she is an aquatic ecologist who conducts research in aquatic community structure.

Dr. Luis Sequeira of the University of Wisconsin said he is a plant pathologist working with bacterial plant pathogens, particularly the Pseudomonads. His research focuses on soil microbiology in general and in the attributes which make certain soil organisms pathogens.

Dr. Margaret Mellon of the Environmental Law Institute said she is a virologist and an attorney. Her area of work is biotechnology regulation.

Dr. Conrad Istock of the University of Arizona said he is an ecologist interested in population genetics. He has worked for many years with insect life history phenomena and has analyzed plant communities from a variety of perspectives. Most recently he has been studying genetic exchange in soil dwelling populations of *Bacillus.*

Dr. Elizabeth Davidson of Arizona State University said she is an insect pathologist working in the area of microbial control of insects. She works primarily with bacteria which kill mosquitoes and black flies.

Dr. McKinney introduced Dr. Elizabeth Milewski, the Executive Secretary of the Subcommittee on Definition of Pathogen, and asked her to describe the objectives of this meeting.

Dr. Milewski said on June 26, 1986, the Federal government published a *Federal Register* document* which described a coordinated framework for regulation of the products of biotechnology. The policy of the Environmental Protection Agency (EPA) is part of the coordinated framework. As indicated in the June 26 *Federal Register,* the Agency will regulate certain products of biotechnology under two of the statutes it administers, the Toxic Substances Control Act (TSCA), and the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Under these statutes, EPA will give regulatory emphasis to three categories of microorganisms:

- Microorganisms which exhibit new characteristics, e.g., microorganisms which are constructed from source organisms from different genera;
- Microorganisms which are released to the environment; and
- Microorganisms which are intrageneric constructs whose parent organism(s) are pathogens.

The June 26, 1986, *Federal Register* contains an interim definition of pathogen as follows:

". . . a pathogen is defined as a virus or an organism (including its viruses and plasmids, if any) that has the ability to cause disease in other living organisms (i.e., humans, animals, plants, or microorganisms). A disease is an abnormal physiological function in an organism, occurring as a consequence of the activity of proliferating microorganisms directly associated with or affecting the host organism, or due to biologically reactive substances such as toxins, antibiotics or growth regulators produced by a microorganism."

Dr. Milewski explained that EPA had formed the Subcommittee on Definition of Pathogen to assist in determining which groups of microorganisms may possess "inherent" risk and should be subject to a higher level of regulatory scrutiny.

Dr. Milewski introduced two EPA personnel who would serve as "resource people" to the subcommittee: Dr. Janet Andersen of the Office of Pesticide Programs (OPP), and Dr. Jane Rissler of the Office of Toxic Substances (OTS). Dr. Milewski said Dr. Ann Huang of OTS would serve as recorder for the meeting.

*Biosafety in Microbiological and Biomedical Laboratories, U.S. Department of Health and Human Services, Centers for Disease Control and National Institutes of Health. 1st Edition. March 1984. U.S. Government Printing Office, Washington, D.C.

**Federal Register* 51:23302-23393.

Dr. Milewski asked Drs. Andersen and Rissler to provide the subcommittee with a brief summary of OPP and OTS perceptions of the issue before the subcommittee.

Dr. Andersen said under FIFRA, any pesticide, including microbial pesticides, sold or distributed in the United States must be registered with EPA. For most pesticides in order to test on 10 or more acres of land or one or more acre of water, an Experimental Use Permit (EUP) must be obtained from the Agency. For most pesticides, no EUP is required for testing on less than 10 acres of land or one acre of water (for the purposes of these minutes "small scale testing"). However, because living microbial pesticides have the potential to persist, disperse and multiply, the Agency felt that certain of these microorganisms should not be tested in the environment on any acreage without prior Agency review. A *Federal Register* published in December 1984* stated this policy in an interim form. The policy was subsequently modified and included in the June 26 *Federal Register.* EPA's policy currently is that prior to small scale testing in the environment these organisms are subjected to either a higher or a lower level of review scrutiny. The determination of review level is based on several criteria. One criterion, described in the June 26 *Federal Register,* is whether the microorganism is a pathogen. Table 1 describes the policy in greater detail.

Dr. Rissler said TSCA also is interpreted to provide two levels of review scrutiny to living biotechnology products released to the environment (Table 1). The lower level review differs from the higher level review in the length of time allotted for the review and in the type and amount of data required.

Dr. Rissler described how the TSCA provisions would be used to provide two levels of regulatory scrutiny to certain biotechnology products. She explained that if a microbe is constructed using source organisms from different genera, that product would be subject under TSCA Section 5 to Premanufacture Notice (PMN) requirements. The PMN review provides a higher level of review scrutiny. If an organism is constructed using source organisms from the same genus, a lower level of review would apply. However, the Agency recognizes that certain intrageneric constructs might warrant a higher level of scrutiny. One provision of TSCA, the "significant new use" (SNUR) provision, will be used to provide a higher level of scrutiny to certain intrageneric products of biotechnology. The June 26, 1986, *Federal Register* states that the SNUR will apply to "pathogens." The definition which describes the organisms subject to the SNUR will circumscribe the scope of EPA coverage within the SNUR.

Dr. Rissler said OTS hopes the subcommittee will provide several approaches to describing the set or subset of microorganisms which would be subject to the SNUR. The Agency would prefer the subcommittee provide a range of approaches to defining the organisms which would be covered by the SNUR rather than a consensus position. EPA will analyze the suggested approaches and any of its own it may develop, select the most appropriate approaches, and develop them into language which will be published for public comment.

Dr. Milewski emphasized that the subcommittee's charge is to suggest approaches to describing the set or subset of organisms which would be subject to the higher level of review scrutiny. The language describing these sets or subsets of organisms would "trigger" this higher level of review. Neither the subcommittee nor the Agency is attempting to define or redefine "pathogen."

Dr. Harschbarger asked what types of organisms are covered by the definition of pathogen published in the June 26, 1986, *Federal Register.* Dr. Rissler replied the definition applies to protists, fungi, bacteria and algae.

Dr. Davidson asked whether nematodes would be covered by the current EPA definition. Dr. Rissler said they would not.

Dr. Milewski said she wished to clarify one point for the subcommittee. In the materials mailed to the subcommittee were excerpts of comments EPA received in response to the definition of pathogen proposed in the June 26, 1986, *Federal Register.* In some of those comments there appears to be some confusion between the United States Department of Agriculture (USDA) definition of "plant pest," which describes organisms covered by the Federal Plant Pest Act, and the June 26 definition of "pathogen" which describes microorganisms to receive greater regulatory scrutiny under EPA statutes.

Dr. Milewski said USDA regulations and implementing mechanisms are different from EPA's regulations and implementing mechanisms. Both agencies are currently in the process of developing definitions to fit their regulations, and expect to coordinate at the staff level and through the committee chartered to provide scientific coordination across the federal agencies, the Biotechnology Science Coordinating Committee (BSCC).

Dr. Milewski asked the subcommittee to focus its attention on describing the organisms which should be subject to the higher level of EPA review, and accept that the Agency will coordinate with USDA. Dr. McKinney said the subcommittee would assume EPA will coordinate with other agencies.

Dr. Richard Perry of the USDA Agricultural Research Service said the Plant Pest Act addresses organisms which are of economic importance to agriculture. The scope of the Plant Pest Act differs significantly from the scope of FIFRA and TSCA. Dr. Perry reaffirmed that USDA and EPA are coordinating their activities at the staff level and through the BSCC.

Ms. Mary Gant of the Office of Science and Technology Policy, the Executive Secretary of the BSCC, reiterated that the BSCC was specifically chartered to coordinate activities between the agencies on scientific issues.

Mr. Thomas Parschley of Ciba-Geigy Corporation urged that the agencies strive for consistency in the definitions used to regulate biotechnology.

Dr. Sequeira asked whether a USDA representative could describe how USDA is addressing the definition of "plant pest."

Dr. Daniel Jones of USDA said the USDA Animal and Plant Inspection Service (APHIS) is in the process of finalizing the proposed rule on plant pests which ap-

**Federal Register* 49:50880

peared in the June 26, 1986, *Federal Register.* APHIS has considered the large number of public comments on the June 26, 1986, *Federal Register* announcement of APHIS' intended regulation. Currently USDA is discussing internally the scope and extent of the regulation.

General Discussion

Dr. McKinney said the charge to the subcommittee is to suggest several approaches which might serve to "trigger" the higher level of review scrutiny. The subcommittee has also been asked to describe the advantages and disadvantages of each suggested approach.

Dr. McKinney said from his perspective the subcommittee was not bound to provide EPA a definition of "pathogen." Rather the subcommittee should provide guidance on which organisms should be subject to the higher level of regulatory emphasis. He asked the subcommittee to consider whether the word "pathogen" should be included in the language describing the set of microorganisms subject to the higher level of review scrutiny.

Dr. Rissler said although the subcommittee is called the Subcommittee on Definition of Pathogen and the *Federal Register* uses EPA's interim definition of pathogen to describe the group of organisms which would receive the higher level of review scrutiny, the subcommittee could suggest EPA not use the word "pathogen."

Dr. Davidson suggested organisms subject to the SNUR could simply be called "SNUR subjects." Dr. Istock agreed.

Dr. Mellon urged EPA not to limit the SNUR to pathogens. The rule ought to be crafted with sufficient flexibility to permit EPA to review other categories of microorganisms without repromulgating the rule.

Dr. Taub suggested two situations in addition to "pathogens" might warrant a higher level of regulatory scrutiny under the SNUR. (1) Situations where large amounts of organisms are introduced in "novel" applications. For example, blue-green algae are being sold as lawn fertilizers. Such a use could have an impact if blue-green algae begin to colonize as the dominant organism in ecosystems where they were not previously the dominant organism. (2) Situations where the ability of microorganisms to degrade chlorinated phenolic compounds such as dioxin is enhanced. The genes encoding enzymes for degrading these compounds are or are related to the genes for enzymes which degrade humic acids such as lignin and cellulose.

Dr. Taub said humic acids are compounds of environmental importance. These substances are relatively inert substrates, and the concern would be that the rate at which they are degraded could be accelerated. She thought organisms which accelerate humic acid degradation might be included under the SNUR. The SNUR could address this concern if the trigger was based on "enhancing" an effect.

Dr. Andersen said OPP had developed an example of the type of language that would be used by the Agency in defining "pathogen." She said this example is a variation on the definition which was published in the June 26 *Federal Register.* It reads in part:

"For the purposes of this policy a pathogen is a virus, viroid, or organism (including its viruses and plasmids, if any) that has the ability to cause an adverse effect on the physiological processes of other living organisms (i.e., humans, animals, plants, or microorganisms). Pathogens may parasitize the host organism directly and cause an adverse disruption or cause an adverse disruption through the production of biologically active substances such as toxins, antibiotics, or growth regulators.

"This policy is not meant to include such organisms involved in commensalistic or mutualistic associations, or opportunistic pathogens. An opportunistic pathogen for this policy is one that can only parasitize severely weakened hosts and will not cause an adverse effect in healthy individuals."

Dr. Taub said this proposed language raises the issue of defining "opportunistic" pathogens and whether these organisms should be subject to the higher level of review scrutiny. She said environmental stresses which are part of normal climactic fluctuation (drought, freezing), weaken populations and render them more susceptible to pathogens which could be described as "opportunistic" in the absence of environmental stress. She thought it difficult to define "pathogen" without considering the environmental context.

Dr. Nielsen agreed the issue of "opportunistic" pathogens should be addressed. What were yesterday's "opportunistic" pathogens are today's killers because of environmental and host changes. For example, several viruses have recently been identified which selectively destroy the immune system. Immunosuppressants are increasingly being used to treat human patients. There are also changes in the environment which predispose populations to disease. For example, increasing concentrations of lead in waterways has led to some degree of immunosuppression in waterfowl and what would have been called an opportunist yesterday are real killers today to these birds.

Dr. Harschbarger added that environmental stresses affect the susceptibility of marine animals to pathogens. For example, the salinity of the Chesapeake Bay decreases in wet seasons and the disease MSX increases. In rivers near sewage outfalls, the number of liver tumors in fish is increasing, probably from exposure to pollutants. He questioned whether the biotechnology industry should be regulated more stringently because other industries are polluting the environment and creating environmental stresses.

In discussing opportunistic versus frank pathogens. Dr. Sequeira stressed that a distinction should be made between the role environmental conditions directly play in pathogenesis and the impact of environmental stresses such as drought and lead poisoning. He offered the example of soft rot bacteria in plants. These bacteria do not attack plant parts unless those plant parts are under semi-aerobic conditions. The fact these organisms do not attack plant parts unless the parts happen to be under semi-aerobic conditions does not mean the organisms are opportunistic pathogens; they are very strong pathogens under appropriate conditions. That type of organism dif-

fers from an organism which under very rare conditions can cause disease.

Dr. McGinnis offered his perspective on the issue of "opportunistic" pathogens. In plant pathology the individual plant is not that critical, but the crop is. The same concept cannot be applied to humans or even to animals. Thus, "opportunists" assume a far greater importance in human and animal pathology than in plant pathology.

Dr. McGinnis said in human pathology, it is difficult to describe a distinction between "opportunistic" and "frank" pathogens. Opportunistic pathogens cannot be defined by describing the host as "severely weakened." He offered the example of a fungus which causes an infection of the brain with a mortality of approximately 95 percent. Although few individuals contract this fungus, careful examination of the patients does not reveal a compromised immune state. He also pointed out that some infectious organisms can suppress the immune system and render the individual more susceptible to organisms ordinarily not efficient at infection. An example of this is pulmonary aspergillosis which can be a sequelae of influenza. These patients would not be described as "severely weakened" or even compromised in the sense of patients receiving immunosuppressive therapy.

Dr. McGinnis said host population demographics change. For example, the immune system declines with age and the number of older individuals in the human population is increasing. Various types of immunotherapies are being developed and their use is increasing. These changes in host population blur any distinction between "opportunistic" and "frank" pathogens.

Dr. Rouse agreed there is a difference in the way plant and mammalian opportunistic pathogens are viewed. Opportunistic plant pathogens do not present the same level of concern as opportunistic mammalian and human pathogens. Opportunistic plant pathogens are not responsible for major economic losses.

Dr. Istock said the words pathogen and parasite are used interchangeably. He questioned whether EPA policy should distinguish between pathogens and parasites.

Dr. Taub asked the subcommittee to consider whether organisms which are not pathogens could become pathogens through genetic modifications. For example, could acquisition of the ability to penetrate the host surface transform nonpathogens to pathogens? Could organisms which have a very low or negligible virulence be converted to frank pathogens by a modification? She offered the example of an increase in virulence in a *Vibrio* following introduction of a plasmid encoding an iron sequestering system.*

Dr. Milewski said the organism Dr. Taub described possessed many of the characteristics necessary for pathogenicity prior to the introduction of the plasmid. She described an experiment in which some traits necessary for pathogenicity were introduced into a nonpathogenic *E. coli* and affected the ability of that organism to infect the urinary tract. Dr. Taub added that introduction of a gene coding for invasiveness had conferred on a nonpathogenic *E. coli* the ability to invade the cells that line the intestine.*

Dr. Sequeira emphasized that *Vibrio* and *E. coli* can be converted to pathogens because they are closely related to pathogens. Organisms which are not closely related to pathogens would not likely be converted to pathogens by such modifications.

Dr. Sequeira said pathogenicity involves a constellation of traits. The probability that a nonpathogen could be converted to a pathogen by introduction of genetic material from a pathogen is very low.

Dr. Sequeira said "new" pathogens generally arise from single gene changes in existing pathogens or from changes in organisms closely related to pathogens. A single gene change will not change a saprophyte to a pathogen. Some degree of scrutiny might, however, be given to the introduction into nonpathogens of genes which code for toxins.

Dr. Istock agreed that many genes are involved in pathogenicity. However, the nearest relative to a nonpathogen may be a pathogen.

Dr. Istock said he supported the comment from the American Society for Microbiology:

"Pathogenic microorganisms are those that cause disease in a host organism, whether the host be human, animal, or plant. This requires an interaction between a pathogenic microorganism and a susceptible individual. There are many factors that are necessary to the expression of pathogenicity. There is communicability, transmissibility, invasiveness, infectivity, and virulence, each of these factors being highly polygenic properties. In other words, each is influenced or created or controlled by many different genes. All pathogens and genetically altered derivatives of pathogens, whether they are deletions, intrageneric recombinants or more distantly related recombinants, should be considered candidates for regulation before introduction to the environment. Some pathogens have the capacity to increase in virulence or change in host range response to a single gene mutation. Some avirulent derivatives of pathogens have the potential to regain pathogenicity by mutation. We agree that such microorganisms need to be examined before release to the environment."

Dr. Istock said Genentech, Inc., comments that "pathogenesis is not an open-ended abstraction, but rather a highly-evolved complex process between a pathogen and its host, an entire array of physiological capabilities that have undergone natural selection in both pathogen and host to establish the fundamental relationships."

Dr. Istock pointed out that the American Indians would not have been comforted by that statement. They had not evolved with the pathogens introduced by European settlers, and were decimated when these organisms were introduced.

*A plasmid associated with virulence in the marine fish pathogen *Vibrio anguillarum* specifies an iron-sequestering system. Jorge H. Crosa. Nature, Vol. 284, p. 566, April 1980.

*Science and the Citizen, Scientific American, 254 (1):70-71, 1986.

Dr. Sequeira said the effect of introducing exotic pathogens is well known in agriculture. Indigenous pathogens can, however, frequently be safely studied.

Dr. McKinney drew the subcommittee's attention to the issue of indigenous and nonindigenous organisms.

Dr. Istock said ecologists have struggled with this issue for years and have developed a whole lexicon of words: "cosmopolitan," "sympatric," "syntopic," and "endemic" have been applied.

Dr. Rissler said at the moment EPA considers geographic point of isolation the basis for designating organisms "indigenous" or "nonindigenous."

Dr. Sequeira thought the concepts of "indigenous" and "nonindigenous" were problematic. Dr. Davidson agreed. She thought it would be very difficult to define "indigenous." Although some of the *Bacilli* she studied were isolated in India, she was certain similar strains could be isolated from her backyard.

Dr. Sequeira said for ubiquitous organisms it will be very difficult to prove that a strain which is isolated in China is also present in the United States although this is likely.

Dr. Davidson questioned whether the word "indigenous" has any utility since modern commerce transports microorganisms worldwide on food and other objects.

Dr. Rouse said as a plant pathologist he would prefer to maintain the distinction between indigenous and nonindigenous microorganisms. Plant pathologists have for many years isolated pathogens and reintroduced them in field trials for research purposes. He did not know of any major problem resulting from such studies. The distinction between indigenous and nonindigenous exempts some indigenous organisms from some levels of review. He was concerned that should the distinction be lost, organisms isolated in the United States would be subject to greater regulatory scrutiny.

Dr. Taub requested a clarification of how EPA's policy deals with indigenous organisms.

Dr. Andersen said if a pesticide is an indigenous pathogen, review would occur when testing is performed on greater than one acre of water or ten acres of land. If the pesticide is a nonindigenous microorganism, testing on any acreage would be reviewed prior to field trial. Dr. Rissler said under TSCA the Agency is not proposing to review commercial small scale testing of organisms constructed using indigenous nonpathogenic source organisms from the same genus.

Indigenous pathogenic nonengineered microorganisms would not be subject to review before small-scale field testing.

Dr. Sequeira asked how the Agency would view a construct where the recipient was an indigenous organism but the DNA donor was nonindigenous. Dr. Rissler said the Agency is currently considering how such a situation would be viewed under the TSCA statutes. In the June 26, 1986, *Federal Register,* EPA indicates such a construct would be subject to the lower level of review. Dr. Andersen said under the FIFRA statutes microbial pesticides which are biotechnology products as defined in the June 26 *Federal Register,* whether indigenous or nonindigenous, will be reviewed under one of the two levels of review prior to small scale field tests.

Dr. Sequeira found the definition of pathogen EPA published in the June 26, 1986, *Federal Register* basically acceptable, although he suggested some minor modifications. He said the definition published in the June 26, 1986, *Federal Register,* because of the emphasis on diseases involving microbes growing in the host, did not cover abiotic diseases. The current Agency definition is constrained by the perception that a pathogen is an organism which infects other organisms, establishes and causes symptoms. There are a number of organisms which cause effects at a distance. An example is the disease of chrysanthemums called strap leaf which is caused by a toxin produced by *Aspergillus* growing in soil. The definition should be broad enough to capture this type of effect.

Dr. Istock agreed the "effect" caused by the organism must be considered. Some microorganisms secrete a toxin which causes an effect at a distance. Some effects are teratogenic, some are oncogenic, some alter immune responses, others cause a shock-like phenomenon, and some interrupt cellular metabolism. These effects should be addressed by the definition of pathogen. In addition, the definition should be flexible enough to encompass the next set of pathogens to evolve or to be identified.

Dr. Davidson pointed out that defining disease as being a consequence of the activity of proliferating microorganisms directly associated with a host organism would exclude from coverage organisms into which the *Bacillus thuringiensis* delta-endotoxin was introduced.

Mr. Parschley said Ciba-Geigy found the definition of pathogen published in the June 26, 1986, *Federal Register* generally acceptable but would suggest some modifications. In particular, the definition of disease should be modified; disease should be described as an *abnormal,* deleterious physiological function in an organism occurring as a consequence of the activity of the proliferating microorganism.

In addition, Ciba-Geigy was submitting a list of general exclusions to the definition which appeared in the June 26, 1986, *Federal Register.* In particular, Ciba-Geigy would like to recommend that certain well-characterized, non-coding regulatory sequences be exempt. Mr. Parschley stated that Ciba-Geigy had prepared a written statement for the record.

Dr. Mellon said the definition in the June 26 *Federal Register* includes "pathogens of microorganisms." She found strange the notion of "pathogens of microorganisms." She could not visualize microorganisms as "unhealthy." She was concerned that if disease was defined as an abnormal physiological state, organisms which are not functioning optimally in certain metabolic pathways could be considered "diseased."

Dr. Andersen said some microorganisms attack other microorganisms. For example, some very important diseases of mushrooms are caused by microorganisms. These types of organisms should be covered by the definition of pathogen. If the word "microorganisms" were deleted from the definition, these organisms would not be covered by EPA regulations.

Dr. McGinnis said he personally found it difficult to think of a fungus which attacks a fungus as a pathogen. These types of organisms in contemporary thought are called mycoparasites.

Dr. Istock thought bacteriophage or plasmids could be considered "pathogens" of microorganisms. Dr. McKinney said microorganisms can be killed by bacteriophage and death is a disease state. Dr. McGinnis agreed.

Dr. Davidson expressed concern about describing plasmids and viruses as "pathogens of microorganisms." She pointed out that most microorganisms carry plasmids or viruses and said this would complicate the regulatory picture.

Dr. Davidson suggested the phrase "including its viruses and plasmids, if any," and the word "microorganism" should be deleted from the June 26 definition.

Dr. Mellon agreed the word "microorganisms" should be deleted from the June 26 definition. She suggested a better approach might be to include as an addendum to the definition, those activities of microorganisms which are of concern. Bacteriophage and plasmids could be described in a more precise manner.

Dr. Taub said she wished to comment on the idea that organisms are healthy or in harmony with their environment when considering a definition of "opportunistic" pathogens. Organisms are not in harmony, they are in fierce competition. Not only are they fighting other organisms, they are adapting to changes in their environment. Algae, for example, go through very dramatic seasonal variations, and disease has a very important influence on their natural cycles.

Dr. Davidson agreed. She said 99.99% of all insect eggs of some insect species never reach the adult stage. That is the natural mortality in the average insect community.

Dr. Harschbarger said in his experience it is very rare to observe a "normal" invertebrate animal in nature. In a whole career one may see one oyster which does not have something wrong with it.

Dr. Istock offered for consideration a modification of the definition published in the June 26, 1986, *Federal Register.* He said:

"A pathogen is a virus or a microorganism, including its viruses, bacteriophages, transposable elements, and plasmids, which can cause debilitating, disfiguring, destructive, or lethal effects upon the anatomy, tissues, organs, physiology, development, or behavior of a host organism. . . ."

Dr. Istock said in this proposed definition, he substituted symptomatic aspects for the word "disease."

Dr. Sequeira suggested a list of organisms might be one approach to describing the organisms which would be subject to a higher level of regulatory scrutiny. The list could either be published or could serve as an internal guidance document to the Agency. He suggested EPA enlist the scientific professional societies in the effort to develop a list.

Dr. Sequeira did not think a definitive list would be feasible or desirable, since it would be very difficult to reflect certain situations on a definitive list. For example, *Rhizobium* is a symbiont of plants, however, some strains of rhizobia produce toxins and are pathogens.

Dr. Istock thought any list should be open-ended in order to encompass the next set of pathogens to evolve or be identified.

Dr. McGinnis said it will be impossible to create a complete listing of microorganisms. Many microorganisms have not been identified. Those microorganisms which have been identified are classified by phenotypic characteristics. This taxonomic classification system will undoubtedly change rapidly in the coming years. Any list that is created should be very flexible to accommodate these changes.

Dr. McGinnis said a listing of fungi is particularly problematic. There are no international committees to make decisions on taxonomic classification of fungi, and a fungus may be known by several names. This situation would lead to confusion in the regulatory mechanism.

Furthermore, because of the way fungal infections are described in the literature, it would be difficult to use a system such as *Index Medicus* to construct a list of fungal pathogens.

Dr. Istock said any trigger based on taxonomy is a problematic approach. Classification at the genus level is not considered to be more than an abstraction for convenience. It is not the taxonomy but the phylogeny which is important. However, it is unlikely that the true evolutionary relationship between microorganisms will be clarified in the near future. Dr. Davidson agreed.

Dr. Mellon suggested it might be possible for EPA to construct a list based on the organisms industry is most likely to use in the next 10 years. These organisms would fall into a relatively small number of taxonomic groups. Dr. Rissler supported this suggestion. Dr. Mark Segal of OTS provided a survey of organisms most likely to be used by industry in the near future.*

Dr. McGinnis thought the CDC-NIH Biosafety Standards for Microbiological and Biomedical Laboratories, which emphasize the degree of virulence, is a reasonable approach to a listing. Dr. Sequeira thought differences in economic importance of various pathogens might be a consideration when developing such a list.

Dr. Davidson said she was attracted to the concept of a list because a list offered several regulatory benefits. She cautioned, however, that problems will arise even if organisms are listed by strain. She offered the following examples. (1) The majority of *Bacillus sphaericus* strains are not pathogenic to insects; however, a subset of strains is insect pathogens. At this time there is no physiological method of distinguishing these strains from the nonpathogenic strains. (2) The only characteristic separating *Bacillus cereus* from *Bacillus thuringiensis* is the ability to produce disease in insects. However, pathogenesis is not a good characteristic for distinguishing organisms taxonomically. (3) How would the list concept deal with nonpathogenic strains which receive toxin genes? How would a strain of *Bacillus subtilis* which had received the gene for *Bacillus thuringiensis* toxin be listed?

*Available in the EPA docket.

Dr. Alan Goldhammer of the Industrial Biotechnology Association (IBA) said the concept of a list approach appealed to him since the status of an organism would be clear. He thought it would probably be easier to devise a list of pathogens than a list of nonpathogens.

Dr. Goldhammer said IBA feels nonpathogens which receive from pathogens coding sequences characterized and shown to be free of pathogenic traits, should not be defined as "pathogens" for regulatory purposes. If these organisms are categorized as pathogens, the regulatory consequences will not only affect their status under FIFRA and TSCA, but will affect their status under other statutes. He was concerned that other agencies would see the descriptor "pathogen" and apply more stringent regulations to control the disposal of waste. Organisms used in fermentations would not be easily disposed of even if they were inactivated, should they be classified as pathogens.

Mr. Parschley said Ciba-Geigy takes exception to the suggestions that: (1) organisms which receive genetic material from pathogens could be defined as pathogens; and (2) an organism be considered a pathogen because it belongs to a species which contains pathogenic strains. An organism should be considered a pathogen if it has the potential to cause disease.

Dr. Taub asked what type of testing requirements would be needed to show that an organism generated by using a nonpathogenic recipient and a pathogenic DNA donor was not pathogenic.

Dr. Sequeira said the issue is how much testing should a submitter perform. Clearly there is a limit on how much testing can be required.

Dr. Rissler said the reluctance of the Agency to require standardized testing prior to EPA review is one of the reasons EPA stated that organisms constructed using genetic material from pathogens would be subject to the higher level of regulatory scrutiny. Testing prior to EPA review would not be required to determine whether the organism was subject to the higher level of regulatory review.

Dr. McKinney said EPA does not mean to imply that introduction of genes from pathogens to nonpathogens renders a nonpathogen pathogenic. Rather EPA will use the definition to trigger review of certain categories of organisms; in this case, organisms constructed using pathogens as the source organisms.

Dr. McGinnis suggested another approach to describing microorganisms subject to the higher level of regulatory scrutiny would be to describe traits which lead to pathogenesis, particularly as these traits relate to host categories. Disease is a dynamic interaction between the pathogen and the host, and the traits of concern to a mammalian pathologist would differ from those of concern to a plant pathologist.

Dr. McKinney suggested a variation on this approach might be to create a list of traits; review might be triggered by the transfer of genes coding for these traits.

Dr. Sequeira agreed the subcommittee might consider a listing of problematic traits. For example, ability to produce toxin would be one trait. The difficulty with this approach is that at this point in time the constellation of traits which result in pathogenicity are not known.

Dr. Taub suggested another approach to describing organisms subject to the higher level of review scrutiny is "use"; how the product will be used or distributed.

Dr. Davidson said an approach based on "host categories" could be a mechanism to implement the trigger for the higher level of regulatory review. Dr. Rissler suggested one approach would be to specify that animal and human opportunistic pathogens are the triggers for review of small scale testing; plant opportunists might trigger review prior to large scale field testing.

Dr. Taub said while a mechanism based on host category would be reasonable for certain groups of organisms (e.g., plants and humans) whose pathogens have received greater study, it would be difficult to implement for organisms such as fish and invertebrates whose diseases and pathogens are not well-studied.

During the general discussion session, Dr. Istock said the exemption of noncoding regulatory sequences in the June 26 *Federal Register* from the higher level of review scrutiny is a problem. Manipulation of these elements may be the most powerful capability of the "new" biotechnology; nonetheless, the current *Federal Register* notice treats modifications involving regulatory sequences as if they were the safest.

Dr. Andersen agreed that elements such as promotors may be the most powerful bits of genetic material. Production of a product may be increased many fold if regulatory elements controlling that product are modified. Such differences in quantity may have a significant impact. For example, toxins which were previously produced in negligibly small amounts might be produced in quantities sufficient to have a noticeable effect.

Dr. Taub said the issue is whether there is a difference between: (1) organisms which produce ten times more of a product; and (2) ten times more organisms. Dr. Davidson said there is a difference between the two cases. In nature the availability of nutrients limits populations. If ten times more organisms are dispersed in a given area, those numbers of organisms will die back to the population level the ecosystem can support. This may not be so in the case where one-tenth as many organisms have ten times greater capacity to produce a specific product.

Dr. Rouse said the Agency at some point may wish to readdress the issue of regulatory sequences and the effect some sequences may have on the rate and amount of synthesis of certain products.

The subcommittee agreed four approaches should be further developed: (1) an approach based on categories of organisms; (2) an approach based on lists of organisms; (3) an approach based on traits that might be of concern; and (4) an approach based on the definition published in the June 26, 1986, *Federal Register.*

Dr. Milewski suggested subcommittee members be assigned to three work groups to write language for the approaches based on categories, lists, and traits. The fourth approach based on the June 26 definition would be developed by EPA staff using comments and the meeting record.

The subcommittee members and EPA support staff were then assigned to work groups. Drs. McKinney, Rouse, Sequeira, Taub, and Milewski would attempt to develop the approach based on traits. Drs. Rissler, Davidson, Harschbarger, and Nielsen would attempt to develop the approach based on categories. Drs. Huang, Istock, McGinnis and Mellon would attempt to develop the approach based on lists of organisms.

After a period during which each work group developed its assignments, the meeting recessed. The following morning, the subcommittee reassembled to describe the language they had developed to describe the three approaches to defining organisms subject to the higher level of regulatory scrutiny.

Description of Approaches

List Approach

Dr. Huang described the list approach developed by her work group. The approach would list strains of microorganisms subject to the higher level of review scrutiny. Pathogens are a well-defined category and could be a class of microorganisms subject to the higher level of review scrutiny.

Dr. Huang said the group thought a list for pathogens and a list for nonpathogens could be created. If an organism were on the pathogen list, it would be subject. Organisms on the nonpathogen list would not be subject. If an organism was not listed, users would contact the Agency to determine whether their products were subject.

A third list might also be considered; that list would consist of organisms for which not enough information is available to determine whether the organism should be on the pathogen or the nonpathogen list.

The lists would serve as a guide and would be illustrative, rather than all-encompassing. The lists should be dynamic, not static. Scientific societies could be asked to assist in creating and revising the lists. EPA would have the final determination on which organisms were entered on the lists.

This approach has several advantages: (1) it would offer industry and the Agency clear guidance on whether a particular organism is subject to a higher level of review scrutiny; a dynamic list would adjust to changes in the science; if scientific societies participated in the effort, lines of communication between interested groups would be reinforced and the burden of generating the list would be shared.

Dr. Huang said a major disadvantage of this approach is the time and effort necessary to construct and maintain a list. A dynamic list could present additional problems; for example, companies may choose to use a specific organism because it is on the list of organisms not subject to the higher level of review scrutiny, only to discover later that due to the fluidity of the list, the organism is now on the list of organisms subject to greater review scrutiny. The opposite situation can also be envisaged. A third disadvantage is the possibility the lists might be "taken as gospel."

Dr. Huang said the group felt organisms should be identified on the lists at the strain level. Some organisms might appear more than once; this might occur, for example, if the lists are subdivided into categories by use or by host.

Dr. Taub said a dynamic list would present some difficulties. The incorporation of advances in knowledge could create confusion: an organism which at some point may not be subject could, because of new information, later be subject to a higher level of regulatory scrutiny.

Dr. Mellon said flexibility has a price. Investigators will have to check the list to determine the status of the organism they are using: the question is "how frequently."

Dr. Istock suggested the Agency only develop a list of organisms known to be "frank" pathogens. Developing a list of the most virulent human and animal pathogens would not be difficult. Difficulties would probably arise in listing "opportunistic" pathogens for reasons described earlier in the meeting. In addition, it would probably be very difficult for the Agency to construct a listing of organisms about which very little is known.

Dr. Sequeira thought it would be very difficult to create a list of nonpathogens since nonpathogens comprise most of the microbial world. He questioned whether the word "nonpathogen" should be used; use of the word suggests "nonpathogens" differ from saprophytes.

Dr. Harschbarger said although he could support a list of pathogens as an implementation mechanism, he could not envisage a list of nonpathogens. Human and plant pathogens have been studied rather extensively but little testing has been done to determine which organisms are pathogenic to invertebrates. In general, the knowledge available in invertebrate pathology is several decades behind the knowledge available for human pathology. Thus, organisms on a listing of nonpathogens might, on further study, be found to be pathogens of invertebrates.

Dr. Davidson agreed. She offered an anecdote concerning the use of *Serratia marsescens* as a marker of water flow in caves. The individuals performing the water flow study considered *Serratia marsescens* a nonpathogen which forms bright pink colonies. Under certain circumstances *Serratia marsescens* is a pathogen of insects and invertebrates, and may impact species well-adapted to cave life (e.g., blind cave fish, white shrimp, etc.).

Dr. Sequeira asked if the group had addressed the issue of whether nonpathogenic organisms receiving genetic material from pathogens would be subject to the higher level of review scrutiny. Dr. Mellon replied the group had not addressed that issue.

Dr. Davidson said this issue should be addressed for some specific manipulations. She offered the example of a *Pseudomonas* which had received the gene for *Bacillus thuringiensis* toxin. If the modified *Pseudomonas* kills insects, it should be considered a "pathogen."

Dr. Rouse said another issue should also be addressed: would a pathogen of fish which is to be used in a terrestrial application be subject to the higher level of review scrutiny.

Dr. Segal suggested some elements necessary to create a dynamic list already exist. The Microbial Strain Data Network is a worldwide system being designed to allow microbiologists access to existing information on microorganisms. Organisms will be listed in the Network on the

strain level. This network, in which EPA participates, could be useful in developing and maintaining lists.

Categories of Organisms

Dr. Rissler then described the approach based on host categories. She summarized the work group's approach as focusing on situations where: (1) deleterious effects could result directly; (2) a less well defined deleterious secondary effect on ecosystems could result; and (3) uncharacterized genetic information could be transferred.

Dr. Rissler said the work group attempted to describe categories of organisms which would be subject to the higher level of review. The group thought toxin producers, mutagens, carcinogens and frank pathogens of humans, animals and plants should be subject. The group also thought microorganisms that have an enhanced capability of degrading naturally occurring, slowly degrading substrates (lignin, cellulose), and organisms that are "significant interruptors" of biogeochemical cycles (nitrogen, carbon, sulfur) might also be subject.

Dr. Rissler said the work group attempted to address the issue of introduction into nonpathogens of genetic material from pathogens. The group proposed that organisms would be subject to the higher level of review: (1) "if they have received by human intervention a gene or genes for toxin production"; or (2) if they are "organisms converted to pathogens by addition of genes" by human intervention; or (3) if the organisms have "received uncharacterized genetic material from pathogens" by human intervention.

Dr. Rissler reported the work group also addressed the issue of indigenous and nonindigenous microorganisms. The work group felt it preferable to subject both indigenous and nonindigenous frank animal and human pathogens to the higher level of regulatory review prior to small scale field testing. Indigenous plant pathogens would not be subject prior to small scale field testing. A chart describing this categorization was presented (Table 3). Dr. Rissler said other categories of organisms could also be evaluated in light of the indigenous/nonindigenous issue, but the work group did not have sufficient time to consider these categories.

Dr. Rissler said her group also attempted to describe some categories of organisms which would not be subject to the SNUR. Most saprophytes, with the exception of those described as subject to to the higher level review, would belong in this category. The work group suggested parasites of microorganisms (protists, fungi, bacteria) be in this category except for those that may be ecologically harmful. Nonpathogenic organisms which receive genes not involved in toxin production or which do not convert the organisms to pathogens would not be subject. Organisms which induce allergenic reactions would not be subject.

Dr. Rissler said in order to implement this approach, frank and opportunistic pathogens and toxin producers would have to be defined. A list of organisms might be useful in implementing this approach.

Dr. Taub said the phrase "environmentally significant substrates" is too broad. She suggested the phrase "naturally occurring substrates that are normally fairly inert and resistant to degradation" be substituted.

Dr. Taub suggested this language could specifically refer to organisms constructed to degrade persistent toxic chemical pollutants. Most of these pollutants are chlorinated phenolic compounds. Organisms possessing the physiological ability to utilize humic acids as substrates will probably be used to develop organisms which can degrade chlorinated phenolic compounds. The concern is that an organism with enhanced degradative capability for chlorinated phenolic pollutants might also have the ability to rapidly oxidize humic acids. Humic acids, under normal circumstances, are degraded so slowly they can be regarded as inert. They form a large part of soil and sediment.

Dr. Sequeira said the majority of soil organisms have some ability to degrade and condense phenols. These abilities might be affected if an organism were genetically modified for some other purpose, but he did not think such changes would lead to organisms which would seriously affect soil structure.

Dr. Taub said in spite of the fact that most soil organisms have these degradative activities, humic acids are only very slowly degraded in nature, as are toxic chemical pollutants. She reasoned that organisms which would have the ability to rapidly degrade toxic chemical pollutants could have very different characteristics than those currently found in nature.

Dr. Mellon asked whether the category consisted of: (1) organisms whose natural ability to degrade humic acids were enhanced; and/or (2) organisms in which other degradative abilities have been enhanced and in which a "spill-over effect" into lignin and cellulose degradative systems had occurred.

Dr. Taub said the language should be directed towards organisms with "enhanced" capabilities to degrade chlorinated phenols.

Dr. Mellon said the language describing this category of organisms should be carefully crafted to indicate that organisms with enhanced ability to degrade chlorinated phenolic compounds would be subject. EPA does not wish to review all organisms possessing genes to degrade lignin and cellulose.

Dr. Segal said lignin and cellulose degradative enzyme systems are among the best studied saprophytic enzymes in the microbial world. Those traits are so well characterized that if EPA were to address this issue, the enzymes which confer the specific traits could be described. He added that these traits are frequently plasmid borne, particularly in the more rapid degradative phenotype.

Dr. Roy Sjoblad of OPP suggested the growth of organisms capable of degrading lignin and cellulose is limited in soil by the availability of nutrients. This suggests organisms with enhanced degradative capabilities are not likely to degrade humic acids to the extent that the structure of soil would be affected.

Dr. Goldhammer urged that EPA not regulate indigenous microorganisms possessing the ability to degrade humic acids or which play a role in biogeochemical cycles.

Dr. Sequeira expressed discomfort with the language referring to "biogeochemical cycles." He pointed out that rhizobia with enhanced nitrogen fixation capabilities have been developed using traditional techniques and applied for a number of years. Use of these organisms has not had an untoward effect on the environment. He strongly suggested the Agency avoid describing this concern. He feared many organisms would be "caught by such a large net."

Dr. Istock said his primary concern is large-scale applications of microorganisms which can affect nutrient flow. He said change in flowrates of environmentally important compounds is an important consideration because such changes can have large impacts. He offered the example of a study on the role of forests in nutrient cycling. The study investigated the suggestion that leveling forests would increase flowrates of water into streams and increase water supply in the northeast United States. However, when the trees were removed, the rate of nitrogen fixation increased and the water no longer passed U.S. Public Health Service standards for potability because of high levels of nitrates.

Dr. Mellon thought this type of concern might not be an issue when small scale field tests are performed. Regulatory review might more appropriately be triggered at the point of large-scale testing or unrestricted use of the product. Dr. McKinney agreed.

Dr. Rissler suggested one approach to describing these two categories of organisms was to create a list of organisms. Dr. Mellon suggested that in contrast to the list approach discussed earlier in the meeting, this list might be inclusive: only organisms on the list would be covered by the SNUR.

Dr. Taub said the group could attempt to describe several specific concerns without using such broad language that many organisms would be covered. In ecology, there is an enormous emphasis on the concept of balance in most systems over an annual or multi-year cycle. If rates change and the system compensates, it's not a problem. If the system is not able to compensate this may present a concern.

Dr. Taub suggested the language be based on "intent"; e.g., did the investigator "intend" to modify a pathway for catabolism of humic acids or to modify flowrates of environmentally important compounds. Dr. Mellon thought such an approach would have to include an objective description of the intended modification.

Drs. Istock, Mellon and Taub proposed the following language:

"Microorganisms which mediate specific changes in rates or pathways of degradation, mobilization or synthesis, within ecosystems. Such microorganisms may be grown in quantity without genetic selection, mutagenesis or techniques or recombinant DNA technology.

"Microorganisms that are specifically developed or modified to change its host range to degrade chlorinated hydrocarbons (e.g., dioxin) will be subject to review. The Agency would be reviewing for secondary effects (e.g , high rates of lignin degradation)."

Dr. Rissler said EPA had hoped to obtain several "triggers" for the higher level of review scrutiny. These triggers would range ideally from a restricted to a fairly broad scope of coverage. The language encompassing enhanced degradative capabilities and biogeochemical cycles interruptors would represent the "broad scope" end of the spectrum. Such a "broad scope" proposal could be published with the others for public comment in the *Federal Register.*

Dr. Davidson asked the subcommittee to address the issue of indigenous versus nonindigenous microorganisms in relation to the approach based on categories.

Dr. Taub asked if the application of large amounts of indigenous microorganisms in the environment would be a concern.

Dr. McKinney said population dynamics might be affected by large scale distribution of microorganisms. He said he would focus on the effect of spreading large amounts of microorganisms in the environment. He would not distinguish between indigenous/nonindigenous or between naturally occurring and organisms "modified by human intervention." If the large scale application of a particular microorganism has an adverse effect it does not matter if the microorganism is nonindigenous or indigenous or how the organism was modified or obtained.

Dr. Taub thought greater consideration should be directed towards organisms modified by human intervention than to the naturally occurring microorganisms.

Drs. McKinney and Sequeira disagreed. Dr. McKinney reiterated his belief that the effect is the important consideration.

Dr. Rissler asked how the subcommittee would view "frank" versus "opportunistic" pathogens under the category approach.

Dr. Sequeira said it would be difficult to describe an opportunistic plant pathogen under the host category approach because the definition of pathogenicity depends on the condition of the host plant. Organisms can use plant constituents as substrates if the plant is impaired, and still not be pathogens. "Frank" plant pathogens are obligate biotrophs, and easier to describe.

Dr. Rouse said opportunistic plant pathogens do not fill Koch's postulates, are not economically important, and are, thus, of less concern. He said this contrasted with human or mammalian pathology. Ordinarily for plants the concern is not for the individual; for mammals generally the concern is for the individual.

Dr. Andersen said "ordinarily" is the operative word since EPA must also consider by law impacts on endangered species. Under the law, consideration goes to the individual whether it be plant or animal.

Dr. Nielsen said the condition of the host plays a major role in disease and "opportunistic" is difficult to define for mammalian pathogens. The host immune system can be suppressed or the individual exposed when the immune system is not fully operative, i e., in neonates or in senescent individuals. Demographics affect the ability of the microorganism to affect the host. This issue has been more fully described in the general discussion.

Dr. Rissler asked whether a sufficient population of compromised hosts exists to justify subjecting opportunistic pathogens to a higher level of review.

Dr. Nielsen said there are more and more of these situations. Natural cycles such as temperature extremes, food availability, drought, and increasing amounts of environmental pollutants, affect host susceptibility.

Dr. Nielsen asked Dr. Davidson if insects would be categorized with plants or with mammals with regard to their response to pathogens. Dr. Davidson said she would categorize insects with plants. Most studies of insect response to pathogens have been on larvae. There is no antibody stimulation of the immune system by antigen as seen in mammals. Insects can be immunized but response is ephemeral and it is not antibody based. Insects probably could not be immunocompromised.

Dr. Davidson said in insect pathology, groups of organisms are known which cannot penetrate the insect cuticle. However, if they do gain access, these organisms cause disease. These organisms might be termed "opportunistic pathogens."

Dr. Davidson said one difficulty with the host category approach is that investigators studying diseases of invertebrates are at the point of describing diseases. They are 20 years behind mammalian pathology.

Traits Approach.

Dr. Rouse then explained the approach using traits of organisms as triggers for review. The language proposed by the work group to illustrate that approach reads as follows:

"For the purpose of the SNUR, microorganisms possessing the following traits are covered:

"1. Microorganisms possessing mechanisms of infectivity, e.g., the ability to recognize a target surface, the ability to attach to a target surface, and the ability to penetrate a target surface.

"2. Microorganisms having a basic compatibility with the host, i.e., the microorganism uses host nutrients and either activates or by-passes host defense mechanisms.

"3. Microorganisms possessing characteristics which affect the host, e.g., toxins, hydrolases, proteases, growth regulators, and induce a deleterious effect. This includes factors that act at a distance, such as toxins.

"4. Microorganisms having mechanisms of dispersal to other hosts.

"5. Microorganisms having an ability to survive by resisting environmental stress or by producing large enough numbers of progeny to successfully maintain its population."

Dr. Rouse said the work group envisaged that possession of some combination of these five traits would cause an organism to be subject to the higher level of review. Some traits could carry an "absolute weight." For example, if the organism could not disperse, it would not be covered. The group did not, however, determine the combination of traits that would make an organism subject to the higher level of review.

Dr. McKinney asked the subcommittee which of the traits listed would have an absolute weight in determining coverage. Dr. Sequeira replied that the ability to cause an adverse effect should have an absolute weight. Dr. Nielsen agreed.

Dr. Rouse said this approach has several advantages as well some disadvantages. The advantages are: (1) The word "pathogen" would not be used; rather possession or absence of certain traits would trigger review. (2) Avirulent forms would not be subject. (3) No distinction between genetically engineered and other organisms would be made. (4) It would be difficult to subvert the system with a set of traits rather explicitly identified. (5) Nonpathogens which had received well-characterized sequences from pathogens might not be covered if the introduced sequences do not code for the traits.

Dr. Rouse said the disadvantages are: (1) The traits of the organism would have to be known. (2) This approach does not address "nuisance" organisms. (3) There is no reference to host range. The subcommittee may wish to consider whether language dealing with host range should be added to the list of traits.

Dr. Sequeira said one characteristic which should be added to the list of traits is "capacity to change host range or virulence by relatively simple mutations."

Dr. Istock said for many organisms host range is controlled by a single gene. He agreed the language should refer to "the ability to change host range." Dr. Rouse also agreed.

Dr. Mellon asked whether genes controlling host range are known and can be easily described. Dr. Sequeira replied that host range genes for plant pathogens are well studied. Dr. Rissler thought for some categories of organisms this type of information would not be available.

Dr. Sequeira thought if the specific intent of the manipulation was to modify host range, the genes controlling host range would be known for the microorganisms being manipulated. Language could be drafted speaking to the "intent" to modify host range by decreasing host range, shifting host range, or expanding host range.

Dr. Sequeira said some consideration might be given to the potential for restoration of function if avirulence is due to a single gene mutation. If it can be shown that the organisms are avirulent by more than a single gene mutation, avirulent forms should not be subject to the higher level of review scrutiny.

Dr. Taub said this approach would not distinguish between opportunistic and frank pathogens. Nonpathogens which had received well characterized sequences which do not cause pathogenicity would not under this approach be subject to the higher level of review. She felt a separate case could be made for transfers involving large amounts of uncharacterized DNA from pathogens, and this might be addressed.

Dr. Rissler asked how investigators would test an organism to determine pathogenicity and host range, particularly if the microorganism is an unidentified isolate. EPA might be asking investigators to gather information in order to determine whether review should occur; this

information might more appropriately be required for the actual review.

Dr. Sequeira said an unlimited number of organisms cannot be tested to determine if they are hosts, but a few species in the area of dispersal could be tested.

Dr. Milewski thought most investigators would choose an organism because of its known traits. She felt very few companies would expend a great deal of money developing an unidentified isolate, and if they did, would probably examine the host range.

Dr. Davidson was concerned that the current language would produce a number of false positives and false negatives. She pointed out that many saprophytes would meet at least one of the criteria.

Dr. Davidson said some pathogens are invasive. For example, *Bacillus larvae* can kill honeybees. The bacteria are phagocytosized in the honeybee gut. The organism does not produce any known toxins. It kills the honeybee by overgrowing the host. This type of organism may not be captured for review under the current language. Viruses generally kill by interfering with the function of the host and the language proposed by the group would not address this type of adverse effect.

Dr. Davidson suggested language referring to "adverse effects such as death" should be added to the language describing the third trait. Dr. Sequeira suggested the traits approach should state that attributes which cause adverse effects are the traits of concern. The subcommittee agreed.

Dr. Sequeira said for an organism to be captured for the higher level of review, it would have to meet at least three criteria. This would meet some of the concern about false negatives and false positives.

Dr. Andersen asked why the fourth trait had been added to the list of traits. She suggested the subcommittee clarify whether the concern was that organisms disperse or that they have the ability to reinfect other individuals.

Dr. Istock said *Trichinella spiralis,* the parasite which causes trichinosis, is an example of an organism which is not able to disperse easily but which adversely affects the host. He questioned whether the third trait would apply to this type of organism if the language only addressed the concern that the organisms can disperse.

Dr. Mellon said one problem with the traits approach was that EPA would have no way to second guess investigators who decided they were not subject to the higher level of review scrutiny. She suggested TSCA section 8(a) reporting requirement might meet some of this concern. Microorganisms which the investigator has determined do not fall under the SNUR would be reported under the 8(a) requirement. In addition, under TSCA, investigators are required to report if in the course of product use it becomes apparent the product poses risks, even if the risk had not been apparent during the testing phases.

Dr. Andersen asked how the group would address the indigenous/nonindigenous microorganism issue under this approach.

Dr. Rissler felt this issue is addressed by the traits approach since only those organisms possessing traits of concern would be subject to the higher level of review. The indigenous/nonindigenous issue is less important under this approach.

Process-Based Approach

Dr. Mellon said one additional approach to implementing a trigger for the SNUR would be "process"-based; i.e., any organism which has been modified using recombinant DNA techniques would be subject to the higher level of review.

Dr. Rissler said a "process-based" approach was first proposed by EPA in 1984. However, many critical comments concerning a "process-based" approach were received and EPA adopted the "product-based" approach described in the June 26, 1987, *Federal Register.*

Dr. Mellon said she recognized the drawbacks of a process-based approach. However, she wished to offer it for consideration because it addresses the public's primary concern in a very direct manner, and is the simplest and most direct response to the issues faced by the Agency.

Dr. Mellon said there are two ways to approach these issues. One method is to subject to greater scrutiny uses of microorganisms which in the past society had determined have acceptable degrees of risk, as well as uses of organisms created by the newly available methods. The other approach is to subject only organisms created by the newly available methods to greater scrutiny.

Dr. McKinney said he saw no reason to treat genetically engineered organisms differently than nongenetically engineered organisms. He would focus on the effect as the primary consideration rather than the method of construction.

Dr. Istock supported Dr. McKinney's argument. He said using traditional techniques such as selective breeding, agriculture had produced many varieties of crop plants. He thought the same could be done with microbes if the time and effort were put into such an endeavor.

Dr. Rissler suggested Dr. Mellon's alternative approach could be published in the *Federal Register* as one of the possible approaches to describing the set of microorganisms subject to the higher level of review.

During the meeting, Dr. Harschbarger stated for the record that investigators who receive EPA permits to test in the environment should submit a type culture to the American Type Culture Collection.

Adjournment

Dr. McKinney said the subcommittee suggested several approaches to implementing trigger mechanisms for the higher level of review scrutiny. While individually these approaches may have deficiencies, collectively they may fill the needs of the Agency.

Dr. McKinney adjourned the meeting at 3:50 p.m. on February 27, 1987.

Respectfully submitted,
Elizabeth A. Milewski, Ph.D.
Executive Secretary

I hereby certify that, to the best of my knowledge, the foregoing minutes and attachments are accurate and complete.

May 7, 1987

Robert McKinney, Ph.D.
Chair
Subcommittee on the Definition of Pathogen

Appendix 2

Subcommittee on the Definition of Pathogen Roster

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The following table summarizes the categories of microorganisms that are given special consideration by the Agency prior to release into the environment as well as the timing of notification and review for these categories of microorganisms. See page 23319 in the June 26 notice.

TABLE 1
Prior Notification and Review of Microorganisms Applies in the Environment (X=Subject to Review; O=Subject to Abbreviated Review)

	FIFRA		*TSCA*	
	<10 Acres	*>10 Acres*	*<10 Acres*	*>10 Acres*
Microbial Product				
I. Genetically Engineered:				
A. *Inter*generic Microorganisms	X	X	X	X
B. *Intra*generic Microorganisms				
1. Pathogen	X	X	X	X
2. Nonpathogen	O	X	O	O
II. Nongenetically Engineered:				
A. Nonindigenous Pathogen	X	X	O	X
B. Nonindigenous Nonpathogen	O	X	O	O
C. Indigenous Pathogen	—	X	O	X
D. Indigenous Nonpathogen	—	X	O	O

Note: *Intergeneric microorganisms* are formed by deliberate human intervention to contain genetic material from organisms in different taxonomic genera. *Nonindigenous microorganisms* are those not native to the continental United States including Alaska, and the immediately adjoining countries (i.e., Canada and Mexico).

TABLE 2
Organism/Use Matrix* — General Use Code

Genus	*A*	*B*	*C*	*E*	*G*	*M*	*N*	*O*	*P*	*R*	*W*
Bacillus	17	97	53	18	30	1	1	3	307	8	83
Clostridium	—	269	204	1	42	1	—	4	14	3	49
Pseudomonas	3	9	44	1	16	14	—	5	57	7	34
Escherichia	2	48	45	10	13	8	5	4	134	1	28
Saccharomyces	—	96	75	1	51	6	—	5	16	—	31
Candida	—	74	58	—	29	2	—	2	35	2	57
Aspergillus	—	13	20	3	2	2	—	—	132	1	72
Kiebsiella	—	75	21	—	14	—	5	7	4	—	24
Alcaligenes	—	—	31	—	6	—	3	8	23	1	55
Aerobacter	—	49	30	—	9	—	—	—	1	—	3
Zymomonas	—	33	25	—	23	—	—	1	—	—	5
Streptomyces	—	15	14	—	1	2	—	—	20	—	33
Kluyveromyces	—	16	15	—	13	—	—	—	10	—	20
Methylosinus	—	1	49	—	—	—	—	1	4	—	8
Thiobacillus	—	—	—	—	—	42	1	10	5	1	4
Penicillium	—	—	—	2	—	2	—	6	23	1	26
Proteus	—	14	8	—	18	—	—	—	2	—	3

General Use

A Agricultural chemicals
B Conversion of biomass
C Industrial chemical production
E Monitoring/measurement/biosensor
G Energy
M Mining/metal recovery
N Nitrogen fixation
O Other
P Polymer/macromolecule production
R Enhanced oil recovery
W Waste/pollutant degradation

*Microorganisms most likely to be used

TABLE 3
Categories Approach
Level at Which Review Will Occur

	Volume of Application	
Frank Pathogens	*Small Scale*	*Large Scale*
Indigenous		
Human	yes	yes
Animal	yes	yes
Insect	no	yes
Non-insect	yes	yes
Plants	no	yes
Nonindigenous		
Human	yes	yes
Animal	yes	yes
Plant	yes	yes

U.S. Environmental Protection Agency Draft Guidelines Greenhouse Containment

On June 26, 1986, the U.S. Environmental Protection Agency (EPA) issued a notice explaining how certain products of biotechnology are subject to review and reporting requirements under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Toxic Substances Control Act (TSCA).*

EPA's biotechnology regulatory policy is particularly dependent on a definition of "release to the environment." The definition is critical to three biotechnology rules to be promulgated under TSCA. Compliance with and enforcement of the FIFRA biotechnology policy is also dependent on this definition.**

A major focus of the EPA policy is oversight of environmental releases of microbial products subject to TSCA and FIFRA. Hence, the definition of release to the environment will determine to a great extent the scope of coverage of EPA's biotechnology policy.

In order to fully implement the policies described in the June 26, 1986, announcement, the EPA must develop a definition of release to the environment for microorganisms.

The description of "contained" greenhouses for procedures involving microorganisms is related to the definition of "release to the environment" and it is likely that the description of contained greenhouse will affect the definition EPA uses to describe release to the environment. In addition, the description of contained greenhouses will be useful as a collation of the practices, procedures and equipment used in greenhouses to control microorganisms.

A subcommittee of the BSAC was convened on March 20, 1987, in Crystal City, VA to assist the Agency in developing the Guidelines (roster follows as Appendix 1). The BSAC Subcommittee on Greenhouse Containment Guidelines was provided with a "strawman" document which it was hoped would be used by the experts in providing a critical discussion of appropriate containment guidelines for procedures which use microorganisms covered by EPA policies.

The "strawman" document was based on the assumption that many of the approaches employed for physical containment in laboratories in the National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules are equally valid for greenhouses. The EPA document was drafted by paraphrasing sections of the NIH Guidelines.

The draft version of the containment guidelines for greenhouses contained in this Report is the product of the March 20, 1987 meeting with minor modifications as suggested by BSAC committee meeting on April 27, 1987.

**Federal Register,* June 26, 1986, Volume 51; 23303 and the article in this *Report,* "The Approach of the U.S. Environmental Protection Agency in Regulating Certain Biotechnology Products."

Environmental Protection Agency Draft Greenhouse Containment Guidelines

Introduction

On June 26, 1986, the Environmental Protection Agency (EPA) issued a *Federal Register* notice explaining how certain products of biotechnology are subject to review and reporting requirements under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Toxic Substances Control Act (TSCA).

In order to fully implement the policies described in the June 26, 1986, *Federal Register,* the EPA must develop certain definitions. Among these definitions is what constitutes "contained" and "released" to the environment when microorganisms are used to perform certain tasks.

The EPA is currently developing several approaches to defining release to the environment. These approaches are based on suggestions offered to the Agency by the BSAC Subcommittee on Definition of Release to the Environment which met in Crystal City, VA on December 11-12, 1986.

The definition of a contained greenhouse is related to the definition of "contained" and "released"; and the guidelines for contained greenhouses will be part of some of the approaches to defining release to the environment currently being developed by the Agency.

As part of its efforts to write guidelines for contained greenhouses, EPA tapped a spectrum of expertise, including the Subcommittee on Greenhouse Containment Guidelines of the Biotechnology Science Advisory Committee (BSAC).

Charge to the BSAC Subcommittee

The Agency expects that this panel of experts will provide a critical discussion of appropriate containment guidelines for procedures which use microorganisms covered by EPA policies and are performed in greenhouses.

The subcommittee has been provided with a "strawman" document which it is hoped will be used by the subcommittee in discussing such guidelines. Working from the assumption that many of the approaches employed for physical containment in laboratories are equally valid for greenhouses, this "strawman" document was developed by paraphrasing the National Institutes of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules.

Physical Containment—Greenhouse

This document describes conditions which would be employed in greenhouses to obtain several levels of containment for procedures involving microorganisms. Combinations of greenhouse operational practices and special greenhouse design can be made to achieve different levels

of physical containment of experimental microorganisms. Four levels of physical containment, designated as GH 1, GH 2, GH 3, and GH 4, are set forth. Each level includes a separate section containing guidance on greenhouse practices and greenhouse facilities.

It is believed that many of the approaches employed for physical containment of microorganisms in laboratories are equally valid for greenhouse situations. Therefore, the National Institutes of Health (NIH) Physical Containment Guidelines (Appendix G of the [NIH] Guidelines for Research Involving Recombinant DNA Molecules) served as a model for the development of this document.

The objective of this document is to provide guidance on how to confine microorganisms in a greenhouse facility and thus to reduce the potential for exposure of persons outside of the greenhouse and the environment to certain experimental microorganisms. The practices and facilities prescribed in these guidelines address the unintentional release of experimental microorganisms out of the greenhouse and into the environment. Each of the major pathways for the release of microorganisms is considered: aerial, in/on solids and liquids, in/on workers, and in/on other biological components such as flying insects. Considerable emphasis has been put on ensuring that, within each GH level, the practices and facilities are as internally consistent as possible with respect to their impact on the magnitude of microorganisms released by each of these pathways.

Physical containment is achieved through the use of practices and special greenhouse design. Emphasis is placed on primary means of physical containment which are provided by greenhouse practices. Special greenhouse design provides a secondary means of protection against the accidental release of microorganisms to the environment. Special greenhouse design is used primarily in facilities in which experiments of moderate to high potential environmental risk are performed. Protective measures for humans working with genetically engineered microorganisms in the greenhouse may be necessary, depending on the microorganism(s) to be used. These measures are not included within the scope of this document. The National Institutes of Health (NIH) Physical Containment Guidelines (Appendix G) should be consulted for guidance on protective measures for humans.

I. Greenhouse Level 1 (GH 1).

I-A. Greenhouse Practices.

I-A-1. Access to the greenhouse is limited or restricted at the discretion of the principal investigator when experiments are in progress.

I-A-2. Personnel are required to read instructions on GH 1 greenhouse practices and procedures and to follow them.

I-A-3. Materials containing experimental microorganisms that are brought into or that are to be removed from the greenhouse in a viable or intact state are transferred in a closed nonbreakable container. A log is kept of viable or intact experimental material entering and leaving the greenhouse.

I-A-4. Experimental microorganisms, including those in or on plants and soil/potting material, must be rendered non-viable before disposal. Methods for rendering experimental microorganisms non-viable are determined by the principal investigator, based on the nature of the microorganisms that are being used.

I-A-5. Standard microbiological procedures are followed for decontamination of contaminated equipment and supplies. Spray or liquid waste from containers used to apply the experimental microorganism shall be decontaminated before disposal.

I-A-6. All procedures are performed carefully to minimize the creation of aerosols and excessive splashing of potting material/soil during the watering process, transplanting, etc.

I-A-7. Persons wash their hands upon exiting the greenhouse.

I-A-8. Insects or other macroorganisms used in conjunction with experiments requiring GH 1 level physical containment shall be housed in appropriate cages. If macroorganisms (e.g. flying insects) are released within the greenhouse at large, precautions are taken to minimize escape from the facility.

I-A-9. A program is utilized to control undesired pests and pathogens, in accordance with local ordinances, the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), Plant Pest Act, and the Noxious Weed Act.

I-B. Greenhouse Facilities.

I-B-1. At a minimum, the greenhouse shall consist of a permanent structure with a continuous covering, located on a site graded to prevent entry of surface runoff, having self-closing, lockable doors.

I-B-2. Each greenhouse contains a facility for handwashing.

I-B-3. Windows and vents that open are fitted with No. 30 mesh (or finer) fly screens.

I-B-4. If intake fans are used, then measures must be taken to minimize the ingress of insects. Louvers on fans shall be controlled so as not to open unless the fan is in operation.

II. Greenhouse Level 2 (GH 2).

II-A. Greenhouse Practices.

II-A-1. Only persons whose presence in the greenhouse is required for program or support purposes are authorized to enter. The principal investigator has the final responsibility for assessing each circumstance and determining who may enter or work in the greenhouse during experiments.

II-A-2. A greenhouse operational procedures manual is prepared and adopted. Personnel are advised of potential consequences of uncontrolled release of the experimental microorganisms to the environment and are required to read instructions on practices and procedures and to follow them.

II-A-3. When experimental microorganisms are present in the greenhouse, a hazard warning sign incorporating the universal biohazard symbol is posted on all access doors.

II-A-4. Materials containing experimental microorganisms that are brought into or are to be removed from the greenhouse in a viable or intact state are transferred in a closed nonbreakable container. A log is kept of viable or intact experimental material entering and leaving the greenhouse.

II-A-5. Experimental microorganisms, including those in or on plants and soil/potting material, must be rendered non-viable before disposal. Methods for rendering experimental microorganisms are determined by the principal investigator, based on the nature of the microorganisms that are being used.

II-A-6. Water that comes in contact with experimental material treated with microorganisms (e.g., runoff from watering plants) is collected and allowed to evaporate or is chemically treated such that release of the experimental microorganism from the greenhouse is minimized.

II-A-7. Standard microbiological procedures are followed for decontamination of contaminated equipment and supplies. Spray or liquid waste or rinse water from containers used to apply the experimental microorganism shall be decontaminated before disposal.

II-A-8. All procedures are performed carefully to minimize the creation of aerosols and excessive splashing of potting material/soil during the watering process, transplanting, etc. Treatment of plants (e.g., spraying) with experimental microorganisms is conducted within an enclosure designed to minimize dispersal of the experimental microorganisms. Treated plants are kept in the enclosure for a sufficient period of time after treatment to allow for settlement of aerosols.

II-A-9. Coats, gowns, smocks or uniforms and shoe covers are worn in the greenhouse. Before leaving the greenhouse this exterior clothing is removed and left in the greenhouse.

II-A-10. Persons wash their hands upon exiting the greenhouse.

II-A-11. Written records of accidents involving inadvertent release or spills of experimental microorganisms are prepared and maintained.

II-A-12. Insects or other macroorganisms used in conjunction with experiments requiring GH 2 level physical containment shall be housed in appropriate cages. If macroorganisms (e.g., flying insects) are released within the greenhouse at large, precautions are taken to minimize escape from the facility.

II-A-13. A program is utilized to control undesired pests and pathogens, in accordance with local ordinances, the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), Plant Pest Act, and the Noxious Weed Act. Particular attention is given to control of flying insects.

II-A-14. Experimental microorganisms assigned to a containment level lower than GH 2 may be tested in a GH 2 greenhouse concurrently with microorganisms assigned to GH 2 provided that the tests are conducted in accordance with GH 2 greenhouse practices. When the experimental microorganisms in use require a containment level lower than GH 2, greenhouse practices will reflect the level of containment required by the highest containment level microorganism being tested.

II-B. Greenhouse Facilities.

II-B-1. At a minimum, the greenhouse shall consist of a permanent structure with a continuous covering, located on a site graded to prevent entry of surface runoff, having self-closing lockable doors.

II-B-2. Passage through two sets of doors is the basic requirement for entry into the greenhouse from the outdoors. The actual greenhouse door must be lockable.

II-B-3. Each greenhouse contains a facility for handwashing.

II-B-4. An autoclave for decontaminating greenhouse materials is available.

II-B-5. Any windows and vents that open are fitted with No. 30 mesh (or finer) fly screens.

II-B-6. If intake fans are to be used, then measures must be taken to minimize the ingress of insects. Louvers on fans shall be controlled so as not to open unless the fan is in operation.

III. Greenhouse Level 3 (GH 3).

III-A. Greenhouse practices.

III-A-1. Only persons whose presence in the greenhouse is required for program or support purposes are authorized to enter. The principal investigator has the final responsibility for assessing each circumstance and determining who may enter or work in the greenhouse during experiments.

III-A-2. A greenhouse operational procedures manual is prepared or adopted. The manual shall include contingency plans to be implemented in the event of a loss of containment. Personnel are required to read instructions on practices and procedures and to follow them. Personnel are also advised of potential consequences of releasing experimental microorganisms to the environment.

III-A-3. When experimental microorganisms are present in the greenhouse, a hazard warning sign incorporating the universal biohazard symbol is posted on all access doors. The sign identifies the microorganisms, lists the name of the principal investigator, greenhouse director, or other responsible person(s), and indicates any special requirements for entering the area.

III-A-4. Experimental materials to be brought into or to be removed from the greenhouse in a viable or intact state are transferred to a non-breakable, sealed primary container and then enclosed in a non-breakable, sealed secondary container which is removed from the facility

through a chemical disinfectant, fumigation chamber, or an airlock designed for this purpose. A log is kept of all experimental material entering and leaving the greenhouse.

III-A-5. No materials, except for experimental materials that are to remain in a viable or intact state, are removed from the greenhouse unless they have been sterilized in an autoclave. Equipment or material which might be damaged by high temperatures or steam is decontaminated by other methods such as gaseous or vapor methods in an airlock or chamber designed for this purpose.

III-A-6. Water that comes in contact with experimental material treated with microorganisms (e.g., runoff from watering plants) is collected and allowed to evaporate or is decontaminated before disposal.

III-A-7. Standard microbiological procedures are followed for decontamination of contaminated equipment and supplies. Spray or liquid waste or rinse water from containers used to apply the experimental microorganism shall be decontaminated before disposal.

III-A-8. All procedures are performed carefully to minimize the creation of aerosols and excessive splashing of potting material/soil during the watering process, transplanting, etc.

III-A-9. A solid front or wrap-around gown, scrub suit or coveralls, shoe covers, and hats are worn in the greenhouse. This clothing is not worn outside the greenhouse and is decontaminated before being laundered.

III-A-10. Persons wash their hands upon exiting the greenhouse.

III-A-11. Written records of accidents involving inadvertent release or spills of experimental microorganisms are prepared and maintained.

III-A-12. A program is utilized to control undesired pests and pathogens, in accordance with local ordinances, the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), Plant Pest Act, and the Noxious Weed Act. Particular attention is given to control of flying insects.

III-A-13. Insects or other macroorganisms used in conjunction with experiments requiring GH 3 level physical containment shall be housed in appropriate cages. If macroorganisms (e.g. flying insects) are released within the greenhouse at large, precautions are taken to minimize escape from the facility.

III-A-14. Experimental microorganisms assigned to a containment level lower than GH 3 may be tested in a GH 3 greenhouse concurrently with microorganisms assigned to GH 3 provided that the tests are conducted in accordance with GH 3 greenhouse practices. When the experimental microorganisms in use require a containment level lower than GH 3, greenhouse practices will reflect the level of containment required by the highest containment level microorganism being tested.

III-B. Greenhouse Facilities.

III-B-1. The greenhouse is a permanent, closed, self-contained structure with a continuous covering which is separated from areas which are open to unrestricted traffic flow. The need to maintain negative pressure should be considered when constructing or renovating the greenhouse. Passage through two sets of lockable self-closing doors is the basic requirement for entry into the greenhouse from access corridors or other contiguous areas. Physical separation of the greenhouse from access corridors, other greenhouses, laboratories or other parts of the facility may also be provided by a double-doored clothes change room (showers may be included), airlock, or similar anteroom arrangement.

III-B-2. The greenhouse facility is surrounded by a security fence or is protected by an equivalent means of security.

III-B-3. The internal walls, ceilings, and floors are resistant to penetration by liquids and chemicals, thus facilitating cleaning and decontamination of the area. All penetrations (plumbing, utilities) in these structures and surfaces are sealed.

III-B-4. Bench tops and other work surfaces that have seamless surfaces which are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat are recommended.

III-B-5. A handwashing facility is located near the greenhouse exit door.

III-B-6. An autoclave for decontaminating materials is available within the facility. A double door autoclave for decontaminating materials passing out of the facility is recommended.

III-B-7. Vacuum lines are protected with high efficiency particulate air (HEPA) or equivalent filters and liquid disinfectant traps.

III-B-8. Windows are closed and sealed. All glazing is resistant to breakage (e.g., double pane tempered glass or equivalent).

III-B-9. An individual supply and exhaust air ventilation system is provided. The system maintains pressure differentials and directional airflow as required to assure flows inward from areas outside the greenhouse.

III-B-10. The exhaust air from the facility is filtered through HEPA filters and discharged to the outside so that it is dispersed away from occupied buildings and air intakes. The filter chambers are designed to allow in situ decontamination before filters are removed and to facilitate certification testing after they are replaced. Air supply filters shall be 80-85% average efficiency by the ASHRAE Standard 52-68 test method using atmosphere dust. Air supply fans are equipped with backflow dampers that will close when the air supply fan is off.

IV. Greenhouse Level 4 (GH 4).

IV-A. Greenhouse practices.

IV-A-1. Only persons whose presence in the greenhouse is required for program or support purposes are authorized to enter. The principal investigator has the final responsibility for assessing each circumstance and determining who may enter or work in the greenhouse

during experiments. Access to the facility is limited by means of secure, locked doors; accessibility is managed by the greenhouse director, local biosafety officer, or other person responsible for the physical security of the facility. Before entering, persons are advised of the potential environmental hazards and instructed as to appropriate safeguards for ensuring environmental safety. Persons authorized to enter the facility comply with the instructions and all other applicable entry and exit procedures. A record of all personnel indicating the date and time of each entry and exit shall be maintained. Practical and effective protocols for emergency situations are established.

IV-A-2. Personnel enter and leave the facility only through the clothing change and shower rooms. Personnel shower each time they leave the facility. Personnel use the airlocks to enter or leave the laboratory only in an emergency. During an emergency, every reasonable effort should be made to prevent the possible transport of viable propagules from containment.

IV-A-3. A greenhouse operations manual is prepared and adopted. The manual shall include contingency plans to be implemented in the event of a loss of containment. Personnel are required to read instructions on practices and procedures and to follow them. Personnel are also advised of potential consequences of release of experimental microorganisms to the environment.

IV-A-4. When experimental microorganisms are present in the greenhouse, a hazard warning sign incorporating the universal biohazard symbol is posted on all access doors. The sign identifies the organism, lists the name of the principal investigator, greenhouse director, or other responsible person(s), and indicates any special requirements for entering the area.

IV-A-5. Experimental materials to be brought into or to be removed from the maximum containment greenhouse in a viable or intact state are transferred to a nonbreakable, sealed primary container and then enclosed in a nonbreakable, sealed secondary container which is removed from the facility through a chemical disinfectant, fumigation chamber, or an airlock designed for this purpose. A log should be kept of all experimental material entering and leaving the greenhouse.

IV-A-6. Supplies and materials needed in the facility are brought in by way of the double-doored autoclave, fumigation chamber, or airlock which is appropriately decontaminated between each use. After securing the outer doors, personnel within the facility retrieve the materials by opening the interior door of the autoclave, fumigation chamber, or airlock. These doors are secured after materials are brought into the facility.

IV-A-7. No materials, except for experimental materials that are to remain in a viable or intact state, are removed from the maximum containment greenhouse unless they have been autoclaved. Equipment or material which might be damaged by high temperatures or steam is decontaminated by other methods such as gaseous or vapor methods in an airlock or chamber designed for this purpose.

IV-A-8. Water that comes in contact with experimental material treated with microorganisms (e.g., runoff from watering plants) is collected and allowed to evaporate or is autoclaved before disposal.

IV-A-9. Standard microbiological procedures are followed for decontamination of contaminated equipment and materials. Spray or liquid waste or rinse water from containers used to apply the experimental microorganism shall be autoclaved before disposal.

IV-A-10. Street clothing is removed in the outer clothing change room and kept there. Complete laboratory clothing, including undergarments, pants, and shirts or jumpsuits, shoes, and hats is provided and worn by all personnel entering the facility. When leaving the laboratory and before proceeding into the shower area, personnel remove their laboratory clothing and store it in a locker or hamper in the inner change room. All laboratory clothing must be autoclaved before laundering.

IV-A-11. Greenhouse accidents involving inadvertent release or spills of microorganisms are reported immediately to the laboratory director and other authorities as appropriate. Written records of such accidents are prepared and maintained.

IV-A-12. Insects or other macroorganisms used in conjunction with experiments requiring GH 4 level physical containment shall be housed in appropriate cages. If macroorganisms (e.g. flying insects) are released within the greenhouse at large, precautions are taken to reasonably preclude escape from the facility.

IV-A-13. A chemical control program is utilized to eliminate undesired pests and pathogens, in accordance with local ordinances, the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), Plant Pest Act, and the Noxious Weed Act. Particular attention is given to control of flying insects.

IV-A-14. Experimental microorganisms assigned to a containment level lower than GH 4 may be tested in a GH 4 greenhouse concurrently with microorganisms assigned to GH 4 provided that the tests are conducted in accordance with GH 4 greenhouse practices. When the experimental microorganisms in use require a containment level lower than GH 4, greenhouse practices will reflect the level of containment required by the highest containment level microorganism being tested.

IV-B. Greenhouse Facilities.

IV-B-1. The maximum containment greenhouse facility consists of either a separate building or a clearly demarcated and isolated zone within a building. The need to maintain negative pressure should be considered when constructing or renovating the facility. Access doors to the greenhouse are self-closing and lockable. Outer and inner change rooms separated by a shower are provided for personnel entering and leaving the facility. A double-doored autoclave, fumigation chamber, or ventilated airlock is provided for passage of those materials, supplies, or equipment which are not brought into the facility through the change room.

IV-B-2. The greenhouse facility is surrounded by a security fence or is protected by an equivalent means of security.

IV-B-3. Walls, floors, and ceilings of the greenhouse are constructed to form a sealed internal shell which facilitates fumigation and is animal and insect proof. These internal surfaces are resistant to penetration and degradation by liquids and chemicals, thus facilitating cleaning and decontamination of the area. All penetrations (plumbing, utilities) in these structures and surfaces are sealed. Sewer vents and other ventilation lines contain HEPA filters.

IV-B-4. Bench tops and other work surfaces that have seamless surfaces which are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat are recommended.

IV-B-5. A double-doored autoclave is provided for decontaminating materials passing out of the facility. The autoclave door which opens to the area external to the facility is sealed to the outer wall and automatically controlled so that the outside door can only be opened after the autoclave "sterilization" cycle has been completed.

IV-B-6. A pass-through dunk tank, fumigation chamber, or an equivalent decontamination method is provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the facility.

IV-B-7. Liquid effluents from sinks, floors, and autoclave chambers are decontaminated by heat treatment before being released from the maximum containment facility. Liquid wastes from shower rooms and toilets may be decontaminated with chemical disinfectants or by heat in the liquid waste decontamination system. The procedure used for autoclaving of liquid waste is evaluated mechanically and biologically by using a recording thermometer and an indicator microorganism with a defined heat susceptibility pattern. If liquid wastes from the shower room are decontaminated with chemical disinfectants, the chemical used is of demonstrated efficacy against the target or indicator microorganisms.

IV-B-8. If there is a central vacuum system, it does not serve areas outside the facility. In-line HEPA filters are placed as near as practicable to each use point or vacuum service cock. Other liquid and gas services to the facility are protected by devices that prevent backflow.

IV-B-9. Windows are closed and sealed. All glazing is resistant to breakage (e.g., double pane tempered glass or equivalent).

IV-B-10. An individual supply and exhaust air ventilation system is provided. The system maintains pressure differentials and directional airflow as required to assure flows inward from areas outside of the greenhouse. Differential pressure transducers are used to sense pressure levels. If a system malfunctions, the transducers sound an alarm. A backup source of electricity is provided to run the air handling equipment if the main power source should fail. The supply and exhaust airflow is interlocked to assure inward (or zero) airflow at all times.

IV-B-11. The exhaust air from the facility is filtered through HEPA filters and discharged to the outside so that it is dispersed away from occupied buildings and air intakes. The filter chambers are designed to allow in situ decontamination before filters are removed and to facilitate certification testing after they are replaced. HEPA filters are provided to treat air supplied to the facility.

Appendix 1

Roster for the BSAC Subcommittee on Greenhouse Containment Guidelines

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EPA Developing Methods To Assess Environmental Release*

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The full impact of the applications of genetic engineering to commercial activities has yet to be felt. The rapid advances of biotechnology companies in the areas of pharmaceuticals, energy production, agriculture, industrial chemicals, paper, food preservation, and insect population management have been paralleled by increasing governmental activity. Regulatory agencies and Congressional committees alike are involved in evaluating research, coordination, and regulatory needs.

The Environmental Protection Agency's (EPA) internal structure separates regulatory responsibility from research efforts. The regulatory offices rely on the Office of Research and Development (ORD) to evaluate and develop methods and protocols for assessing potential environmental and human effects of industrial products. ORD research is oriented toward developing new procedures as needed and applying existing knowledge to improve the Agency's analytic capability.

The EPA has invested considerable research effort—intended to meet regulatory needs—toward developing methods for assessing the environmental effects of genetically engineered microorganisms (GEMs).

EPA's Involvement in Biotechnology

EPA's biotechnology activities began with a series of three workshops—held in 1978, 1979, and 1980—during which the role of EPA vis-à-vis the potential biotechnology industry was explored.[1] Industrial, academic, and government scientists reached three major conclusions. The Agency should:

1. Examine sewage systems and aerosols as vectors for the dissemination of genetically engineered microorganisms;

2. Evaluate the potential for adverse environmental effects of engineered organisms; and

3. Develop a research plan for studying the release, survival, colonization, potential for genetic exchange, dissemination, and effects of released engineered organisms.

The ORD proceeded to survey the emerging applications of biotechnology to agriculture and industry. Preliminary investigations centered on the fate, survival, and accidental release of engineered microbes, as well as the efficacy of biological containment of GEMs in sewage. Much of the data from these studies has been published in both government reports and articles[1,2,3] and was summarized at a risk assessment symposium.[4]

ORD also began an effort to identify specific Agency capabilities and research needs relative to applying the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Toxic Substances Control Act (TSCA) to biotechnology products. A series of in-house workshops culminated in a conference held at Coolfont, WV (April-May 1984).[5] The conclusions reached at the conference were used by the Agency to produce an overall research plan and to identify data gaps for future work. The major conference conclusions were:

1. To concentrate on environmental effects, including methodology for estimating fate and effects of engineered microbes; and

2. To adopt a case-by-case approach for evaluating the release of GEMs.

To implement the program, the EPA allocated $1.5 million in FY '85, $4.5 million in FY '86, and $4.8 million in FY '87.

EPA's overall research plan (Figure 1) focuses on risk assessment and includes development of methods and protocols for laboratory studies, evaluation and modification of methodology in microcosms, evaluation of the use of microcosm data in terms of equivalence to field data, and preparation of risk assessment guidelines.

Several of the Agency's laboratories and a number of universities are involved. The Corvallis, OR, and Gulf Breeze, FL, environmental research labs focus on terrestrial and aquatic ecological assessment problems, respectively. The Health Effects Research Laboratory (Research Triangle Park, NC) focuses on methods to estimate health effects and guidelines for risk assessment. The Cincinnati, OH, laboratory is focused on accidental release and problems associated with containment and decontamination. The Agency has facilities for P-3 level containment and for contained microcosm studies. Table 1 summarizes the range of topics being investigated by extramural researchers funded by ORD.

Eighty percent of biotechnology risk assessment funds are allocated to environmentally oriented research, reflecting the area of deepest uncertainty. Health and industrial issues are closely related to topics covered by the Agency's continuing research on microbial pest control agents (MPCAs) and industrial exposures.[5] Therefore, the following information on the EPA's current research program is biased toward environmental issues.

Detecting, Identifying, and Enumerating GEMs

Adequate assessment of environmental and public health impacts of GEMs requires reliable methodologies for identifying and enumerating microorganisms in complex environmental samples and tissues. The methods must address the analytical and operative criteria required for any monitoring program. That is, they must be sensitive and specific so as to differentiate an extremely low level of GEMs from the complex background of indigenous organisms. The methods must not only be accurate and reproducible, but widely applicable as well. GEMs may be found in samples from ecosystems that differ markedly, such as leaf surfaces and freshwater reservoirs. Finally, the methods have to be feasible. They must be cost-effective and technically straightforward. Although some

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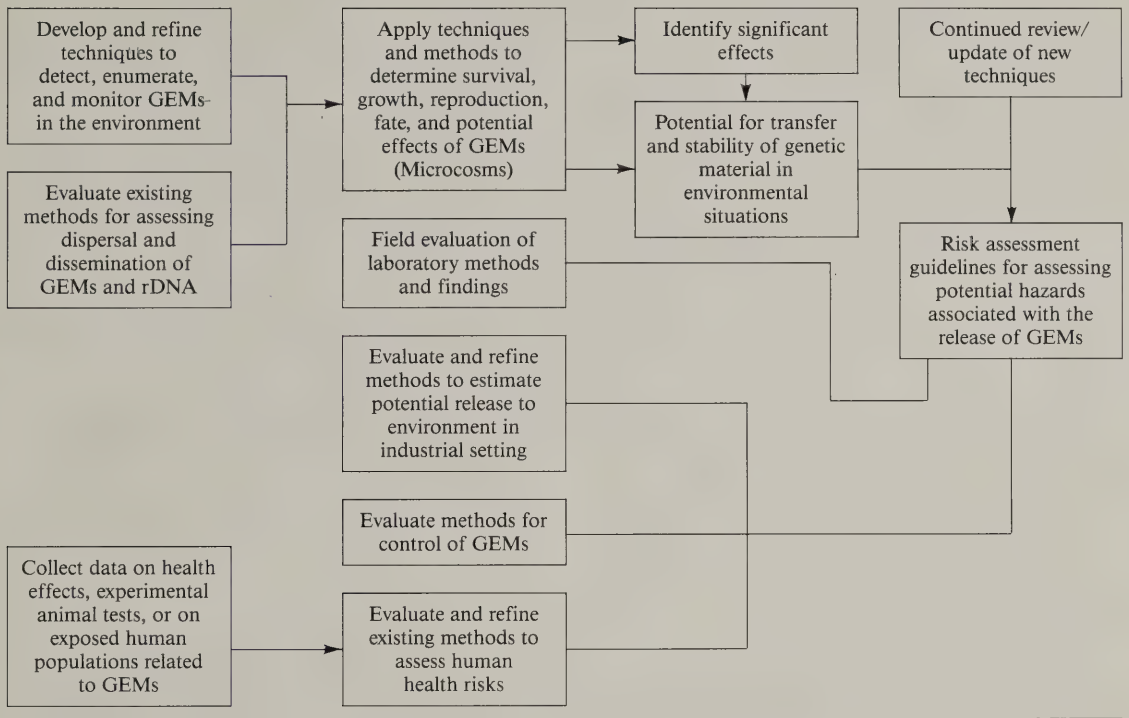
FIGURE 1
EPA's Biotechnology Risk Assessment Research Program



current methods may be directly applicable (Table 2), others will need modification, and all must stand up to comparative evaluation. Each method has its specific strengths and weaknesses, but each must be reliable in its ability to quantitatively enumerate GEMs.

The methods currently under evaluation for detecting, identifying, and enumerating GEMs in environmental samples are summarized briefly below and outlined in Table 2. (For a full description, see "Detection Technology: the Key to Environmental Biotechnology," *Bio/Technology* **4**:419, May '86.)

Selective Media

This technique is very sensitive, since the medium selects against organisms other than the one of interest. Including more than one selective agent in the medium can increase the precision of detection. The effectiveness of selective media, however, depends on the microorganism's abilities to grow and to express specific genes whose functions are needed for identification. Both of these characteristics may be inhibited by selective agents. In addition, in the natural environment where physiological conditions are less than optimal, some microorganisms enter "nonculturable" stages. DNA elements are lost and the expression of specific gene systems may cease or change dramatically.[14]

Fluorescent Antibodies

Specific organisms in environmental samples can be detected directly by reacting the samples with fluorescent monoclonal antibodies to purified cell wall components. This method has already been used extensively to differentiate between species of *Rhizobium*[7], to detect aquatic microorganisms in "nonculturable" life stages[14] and to differentiate between Baculovirus species. However, the inability to differentiate between living and non-living microorganisms is a limiting factor. Also, the method relies on microscopic observations. Approaches for modification of this technique include sample concentration procedures[7], flow cytometry[14], and enrichment procedures.[15]

Hybridization with DNA Gene Probes

This method relies on the formation of base pairs between homologous nucleic acid sequences, permitting identification of specific sequences homologous to the labeled DNA probe. The method is used for the detection of specific organisms in environmental samples[10,12] and pathogenic organisms in water systems and food products.[16] Since the method is independent of gene expression, identification is assured even though the selected trait may not be expressed. The present method, however,

TABLE 1
Extramural Research Funded by ORD

Recipients

Alexander, M., ***Cornell University***
Fate in natural environments for new genotypes developed by genetic engineering

Crawford, D., ***University of Idaho***
Effects of released recombinant streptomyces on the microbiology and ecological processes of a soil ecosystem

Colwell, R., ***University of Maryland***
Survival, modification and effects of genetically engineered microorganisms released to the aquatic environment

Geault, M., ***Drexel University***
Exchange of recombinant DNA in sewage

Lensky, R., ***University of California, Davis***
Genetic factors influencing the ecological fate of two model recombinant microorganisms

Lindow, S., ***University of California, Berkeley***
Determination of the fate and effects of released recombinantly generated microorganisms and their parental strains compared with measurements made in contained environments

Miller, R., ***Loyola, University of Illinois***
Genetic transfer in aquatic environments

Morita, R., ***Oregon State University***
Impact of energy controlled survival on the stability of plasmid DNA in genetically engineered microorganisms

Olson, B., ***University of California, Irvine***
Evaluation of novel methods for detection of recombinant DNA in the soil environment

Sayler, G., ***University of Tennessee***
Genetic approaches for determining persistence and effects of introduced species

Stahl, D., ***University of Illinois***
The use of rRNA sequences to characterize natural microbial populations

Recipients

Stotzky, G., ***New York University***
Fate of genetically engineered microbes in soil and effects on ecological processes

Suslow, T., ***Advanced Genetic Sciences***
Ice nucleation and bioluminescence as sensitive tools for detection of GEMs

Tiedje, J., ***Michigan State University***
Methods for assessing genetically engineered microbes in soil

Van Voris, P., ***Battelle Memorial Institute, Northwest***
Evaluation of terrestrial microcosms for assessing fate and effects of genetically engineered microorganisms on ecological processes

Walter, R., ***Florida State University***
The construction of *Bacillus subtilus* strains containing a marker sequence allowing quantitative enumeration in environmental cultures

Yoder, O.C., ***Cornell University***
Effects of foreign genes on virulence of pathogenic fungi

Visiting Scientists

Chakrabarty, A.
Microbial geneticist, Distinguished visiting scientist

Chatterjee, D.
Microbial geneticist, National Research Council Associate

George, E.
Molecular Biology, NRC Associate

Olsen, R.
Microbial geneticist, Distinguished visiting scientist

Stotzky, G.
Microbial ecologist, Distinguished visiting scientist

Walter, D.
Microbiology geneticist, NRC Associate

is not sensitive enough to pick out a few GEMs from the indigenous community. Kuritza and Salyers[17] have concluded that more than five percent of the DNA in a sample must react with the probe before detection is possible.

Ribosomal RNA (rRNA) Sequencing and Fingerprinting
Ribosomal RNA sequences are personal monograms of bacterial species; they have been used to construct phylogenetic trees[18] and to examine relatedness among *Vibrio cholerae* isolates.[13] Although rRNA sequencing is laborious and test results must be confirmed by other procedures, modification and combination of this method with other detection procedures could provide a highly specific and sensitive methodology.

Data Comparability
Because the protocols developed will be used by numerous laboratories and industrial concerns, establishing the comparable reliability and efficiency of the various methods is essential to the success of the program. To this end, a set of "bench mark" reference organisms has been constructed. They are being used by all the laboratories involved in EPA's biotechnology program. The "bench mark" organisms are bacterial strains containing selected

TABLE 2
Methods Being Evaluated/Modified/Tested for the Detection, Identification, and Enumeration of GEMs in Environmental Samples

Method of Detection	*Strength*	*Weakness*	*Use in Microbial Ecology*[1]
Selective Media	High sensitivity Operationally feasible	Requires growth on laboratory growth media	Gerhardt, 1981[6]
Fluorescent Antibody	High specificity Direct detection in environmental samples	Low sensitivity No differentiation between dead and live cells	Bohlool and Schmidt, 1980[7]; Irby *et al.*, 1985[8]; Huang *et al.*, 1985[9]
DNA Gene Probes	Detection is based on the genotype rather than on the phenotype of GEMs Direct detection in environmental samples	Low sensitivity	Barkay *et al.*, 1985[10]
rRNA Sequencing and Finger Printing	High specificity Very concise	Low sensitivity Low operational feasibility	Stahl *et al.*, 1985[11]; Loh *et al.*, 1982[12]; MacDonell and Colwell, 1984[13]

[1]References demonstrate the use of these methods in microbial ecology research.

plasmids and transposons, developed by Ron Olsen (University of Michigan). In addition to serving as quality assurance measures (i.e., allowing standardization of procedures across laboratories), the reference organisms will help determine which method of detection is most appropriate for a particular GEM and for its environment.

Application of Methodology

As the methods become available, program scientists can begin to address questions pertaining to genetic transfer, stability, and effects on the ecosystem. Ecosystem response to the release of GEMs depends, in part, on the stability of the recombinant DNA (rDNA) in the environment. Even if the gene products are capable of adverse ecological effects, the rDNA's instability or lack of expression in the environment would result in reduced risk. On the other hand, horizontal gene transmission from GEMs to indigenous flora may affect the ecosystem if the exchange results in a more stable inheritance and expression. Critical evaluation of existing data indicates that genetic material is lost, yet also transmitted in the environment.[19]

Current studies are listed in Table 1 and described below. These studies include efforts to estimate gene transfer rates in the environment and effects of ecological factors on gene expression and stability, as well as the impact of GEMs on ecological processes.

The significance and frequency of gene transfer in the environment will be evaluated by observing gene transfer frequencies under optimal laboratory conditions, in sterile and non-sterile aquatic and terrestrial systems, in microcosms, and in contained chambers placed in the environment. These studies will also examine the validity of using laboratory experiments to simulate *in situ* transfer events.

The effect of ecological factors on genetic stability will be determined by tracking specific genes while varying parameters known to affect microbial activity (e.g., nutrient concentrations, particulate matter, pH, Eh, temperature, salinity). A comparison of samples from around the world should shed some light on the effect of soil type on detection, survival, and growth of GEMs. Parameters found to affect genetic stability in *in vitro* experiments will then be studied in soil and aquatic microcosms.

The role of positron-effect on genetic stability will be determined by following the fate of rDNA inserted in different parts of the microbial genome. The stability of recombinant genes incorporated into plasmids, transposons, and chromosomes will be tested in host systems that are routinely manipulated by the biotechnology industry for environmental application. The location and organization of rDNA in transconjugates (transductants and transformants) will be determined using restriction

enzymes, fingerprinting, and Southern hybridization. These studies will identify some of the molecular characteristics that affect stability and spread of rDNA to indigenous flora.

Factors affecting gene expression in the environment will be studied by comparing the genotype with the phenotype of GEMs in microcosms. It is likely that GEMs will be released into environments where their activities are required, and therefore the environment will either naturally support or will be induced to support genetic stability. Research efforts will focus specifically on identifying those factors that may act as positive sectors for the stability and expression of rDNA.

Ultimately, the results of laboratory studies and field validation efforts will be integrated into mathematical models whose validity could be confirmed by field observations. Historically, this approach has been used to predict the environmental fate of chemical pollutants.[20] Existing models for gene transfer among bacterial populations[21] could be modified to describe these events in natural environments.

Health Effects Research

Most of the EPA's biotechnology-oriented health effects research is a direct extension of the 10-year-old Microbial Pesticides Control Agents program, aimed not only at identifying and characterizing these agents, but also at determining their safety vis-à-vis non-target species. From 1974 to 1980, the Health Effects Research Laboratory's effects were directed toward developing techniques that could be used by pesticide manufacturers and the EPA to identify Baculoviruses.

Early studies focused on characterizing the viruses and developing monitoring methods to detect and identify them. The results demonstrated that Baculovirus species yield characteristic and unique DNA restriction endonuclease fragments and SDS polyacrylamide gel patterns. This provided an identification procedure, so that proteins or DNA from an unknown Baculovirus source can now be compared to a known reference.[22,23,24,25] Confirmatory techniques using DNA hybridization procedure[25] and radioimmunoassays[26] provide supportive identification methods.

There are more complex microbial pest control organisms available—including Bacillus strains, fungi, and protozoans such as the microsporidium *Nosema locustae.* Restriction endonuclease fragment analysis is not as useful for identifying these organisms because of the size and complexity of their genomes. Emphasis has shifted to techniques employing specific antibodies.

Enzyme-linked immunosorbent assays (ELISA) incorporating monoclonal antibodies have been used to characterize and differentiate microsporidian species, as well as the insecticidal and putative hemolytic components of strains of *Bacillus thuringiensis* subspecies *israelensis* and *kurstaki.*

EPA researchers have cloned the genes for several of the *B. thuringiensis* proteins, and are characterizing them in combination with the ELISA studies. It is anticipated that biological activities reported in the literature can be assigned to specific proteins and the specific locations of their genes determined. The role of plasmids in the expression of the genes controlling toxin crystal formation will be examined by transfer of plasmids into non-crystal forming bacteria by conjugation and by transpositional mutagenesis (using a temperature-resistant plasmid).

Information on the ability and rate of genetic transfer as well as the potential for expression of the transferred rDNA will be used to evaluate the potential for adverse mammalian health effects of Baculoviruses and *Bacillus thuringiensis.*

Quantitative procedures are essential for developing an understanding of the mechanisms associated with survival, colonization, and effects of introduced microbes. With environmental information and data from laboratory effects tests, predictive models could become an integral part of the regulatory decision-making procedure.

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

March 3, 1986

Honorable Lee M. Thomas
Administrator
U. S. Environmental Protection Agency
401 M Street, S. W.
Washington, D. C. 20460

SAB-EC-86-014

OFFICE OF
THE ADMINISTRATOR

Dear Mr. Thomas:

The Science Advisory Board's Executive Committee transmitted, on February 7, the final report of its Study Group on Biotechnology which evaluated the Agency's research and regulatory programs for this evolving technology. Among the conclusions reached by the Study Group was the need for a broader research effort to improve the Agency's capability for risk assessment of genetically altered organisms, general endorsement of the preliminary policy statement for testing, and the need for the development of additional test protocols to comply with current and future regulatory requirements.

The Study Group also recommended the creation of an advisory committee to provide a continuing independent review of the technical adequacy of risk assessments prepared by the Agency before granting experimental use permits. In judging the need for a separate committee, the Study Group and the Executive Committee assumed that confidential business information (CBI) would constitute a significant portion of the technical data submitted by individuals and organizations seeking an EPA permit, and that the number of permit petitions would grow significantly in future years. Because the Science Advisory Board is a public advisory body whose members are not generally cleared for CBI data, it is the Board's recommendation that the new biotechnology scientific advisory committee should be separate from the Science Advisory Board. It would be useful, where circumstances warrant, to have overlapping membership between this committee and the Science Advisory Board. In addition, we recommend that the committee chair serve as a member of the SAB Executive Committee.

The Executive Committee discussed these and other issues with the Assistant Administrator for Pesticides and Toxics Substances, John Moore, during its January 30-31 meeting. Dr. Moore's conception of the role of this committee, as it would function under the umbrella of the proposed interagency Biotechnology Science Coordinating Committee, closely matched the views of the Executive Committee. In addition, there was consensus that the Science Advisory Board should maintain a parallel standing